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ON

THE NEPHROTIC SYNDROME

EDITED BY

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INTRODUCTION

The Eighth Annual Conference on the Nephrotic Syndrome, held at the Albert Einstein College of Medicine, Yeshiva University, New York City, on Friday, October 12, 1956, convened at 9:30 A.M.

DR. HENRY BARNETT: I want to welcome all of you here. We are fortunate this year to have with us a number of people from outside the United States who are interested in our common problem.

Since some of you are attending this seminar for the first time, I thought I might say just a word about its origin. It was conceived I think in May nine years ago, at Atlantic City, when Dr. Metcoff suggested to me that maybe it would be a good idea for a group of us to get together for such a seminar. The first one was held the following year at the New York Hospital. Since then, as many of you know, we have met each year, this being the eighth such seminar. We believe the seminars have been very valuable to us. Many of the people here have been present at all of them and it is particularly gratifying to me to be host this year and to invite you to see our new school.

I would like to ask Dr. Abraham White, who is Professor of Biochemistry at our School and Associate Dean, if he would greet you.

DR. WHITE: In the unavoidable absence of Dean Kogel, it becomes my very pleasant duty on behalf of the Albert Einstein College of Medicine and the Bronx Municipal Hospital Center to welcome you here for this symposium.

The word "symposium", although you prefer to call this a seminar, is derived from the Greek word "symposion" meaning a drinking party, or, if you prefer, from the French "syn" meaning with and "posis", with drinking. In ancient Greek times symposia were occasions on which there was great feasting, drinking, and music but with great emphasis upon informal conversation and the free interchange of ideas. Although we cannot supply you with the accoutrements of the symposium in the traditional or classical sense, we do know that you will have a great feast of experimental data and ideas.

It is a very great pleasure to have you all here. We hope that individually and collectively you will come back many times.

DR. BARNETT: Thank you! As many of you know, the proceedings of these discussions are published and have been of great value to investigators and other physicians throughout the country who cannot attend the meeting because by necessity we need to keep these sessions small and informal.

The program for today and tomorrow is known to you. We have not really devoted an intensive discussion to the old and newer data on the transport of macro and micro molecules. Our entire non-clinical discussion this year will be devoted to these subjects.

We plan to start with data concerning the transport of large molecules and for this session Dr. Norman Kretchmer of the New York Hospital will serve as Chairman. The group participating in this discussion I think had a preliminary meeting yesterday and we look forward to their discussion. Dr. Kretchmer, would you take over?

I. TRANSPORT OF MACROMOLECULES

CHAIRMAN KRETCHMER: The topic for this morning will be a discussion of the transport of macromolecules which will be limited to the transport of proteins.

As in years past this topic has always been given considerable thought and we thought a discussion of this kind should start on a very fundamental level. I believe that there is no one better qualified to initiate this discussion than Dr. Jean Oliver.

A. Cellular Mechanisms of Protein Metabolism in the Nephron

DR. JEAN OLIVER: I have made an attempt to boil down to essentials the very considerable amount of evidence that has accumulated concerning the way the nephrons handle certain larger aggregates, especially the proteins.

It was Susuki who in 1913[1] first accurately described the absorption and concentration in particulate form by the renal cells of the dyes carmine and trypan blue. Any doubt as to the accuracy of this localization of the process in the proximal convolution and as to its gradient of accumulation was removed two years later by v. Möllendorf[2] and by myself[3], who demonstrated these phenomena in isolated, dissected nephrons.

You are all familiar with the long series of studies which followed, in which Lambert[4], Gerard and Cordier[5], Randerath[6], Smetana[7] and others showed that after experimental injection of physiological and labeled protein droplets formed in the tubule cells of the proximal convolutions. The droplets were assumed to be protein absorbed from the fluid in the tubule lumen, to some the idea of "storage" seemed appropriate and hence the German word "Speicherung". As will become apparent, the

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[1] Susuki, T., Zur Morphologie der Nierensekretion unter Physiologischen and Pathologischen Bedingunen. G. Fischer, Jena, 1912.

[2] v. Mollendorf, W., Die Dispersität der Farbstoffe, inre Beziehungen zur Speicherung und Ausscheidung in der Niere Anat. Hefte, 53:87, 1915.

[3] Oliver, J., The Histogenesis of Chronic Uranium Nephritis with Especial Reference to Epithelial Regeneration. J. Exp. Med. 21:425, 1915

[4] Lambert, P , Sur L'Existence d'un Gradient de Permeabilité dans les Nephrons Ouvert des Urodiles. Compt. Rend., Soc., Biol., 114:1370, 1933.

[5] Gerard, P. and Cordier, R., Esquisse d'une Histophysiologie Comparée du Rein des Vertébrés. Biol. Rev., 9:110, 1934.

[6] Randerath, E., Die Entwicklung der Lehre von den Nephrosen. Ergeb. d. allg. Path. u. path. Anat., 32:91, 1937.

[7] Smetana, H., The Origin of Colloid and Lipoid Droplets in the Epithelial Cells of the Renal Tubules. Amer. J. Path., 18:1029, 1942.

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[7] Smetana, H., The Origin of Colloid and Lipoid Droplets in the Epithelial Cells of the Renal Tubules. Amer. J. Path., 18:1029, 1942.

choice of this term was perhaps unfortunate as the ultimate *result of the process is* the reverse of conservation or "storage" but is rather "disposal". If the period of intracellular accumulation is at times protracted, it is only because the cell cannot *promptly get rid of* a substance that is difficult of disposal.

A more popular term, "Athrocytosis", though accurate enough in a descriptive sense, has *resulted in the verbal hypostatation of an undetermined* cytological process which has apparently been accepted as "summing up" if not explaining the handling of proteins by the nephrons. I can recall occasions when on pointing out that proteins are filtered and reabsorbed by the renal cells, I was assured that this was not "tubular reabsorption"; it was "athrocytosis", and the discussion passed to other matters which were at that time regarded as more proper to the field of renal function. As we know now, and as will doubtless be a matter for discussion in this conference, "threshholds" of protein reabsorption exist and can be measured by the same technical methods of *clearance and* T_m *as are used for other urinary substances. The ultimate cellular* mechanisms concerned in these transportations of various substances across cell membranes are doubtless very different, but the fact remains that they are similar to other effects which the nephrons produce in modifying the plasma which circulates about them. They are therefore renal functions.

My interest in intracellular droplets began in *1913* when *Thomas Addis* gave me some kidneys from rabbits on which urea ratios had been done and in which the cells of the proximal convolutions contained droplets. It is of some interest and indicative of the state of opinion in that distant time to note that attempts to correlate their presence with some aspect of the secretion of urea failed. During the last war our work on the effects of various blood substitutes, including proteins, again forced droplets to my *attention. It was during these investigations that the introduction of phase microscopy* led to a simple observation from which the recent work [8, 9, 10, 11, 12, 13, 14, 15] of the group at *New York State University derived.*

[8] Oliver, J., The Structure of the Metabolic Process in the Nephron. J. Mt. Sinai Hosp., 15:173, 1948.

[9] Oliver, J., MacDowell, M. and Lee, Y.C., Cellular Mechanisms of Protein Metabolism in the Nephron. I. The Structural Aspects of Proteinuria; Tubular Absorption, Droplet Formation and the Disposal of Proteins. J. Exp. Med., 99:589, 1954.

[10] Oliver, J., Moses, M. J., MacDowell, M. C. and Lee, Y. C., Ibid. II. The Histochemical Characteristics of Protein Absorption Droplets., J. Exp. Med., 99:605, 1954.

[11] Lee, Y. C. Ibid. III. The Histochemical Characteristics of Amino Acid Droplets. J. Exp. Med., 99:621, 1954.

[12] Kretchmer, N. and Dickerman, H. W., Ibid. IV. The Partition of Succinoxidase and Cytochrome Oxidase Activities in the Cells of the Proximal Convolution of the Rat After Intraperitoneal Injection of Egg White. J. Exp. Med., 99:629, 1954.

[13] Kretchmer, N. and Cherot, F. J. Ibid. V. The Intracellular Partition and Incorporation into Protein of Intravenously Injected L-Lysine. J. Exp. Med., 99:637, 1954.

[14] Straus, W. and Oliver, J. Ibid. VI. The Immunological Demonstration of Egg White in Droplets and Other Cellular Fractions of the Rat Kidney After Intraperitoneal Injection. J. Exp. Med., 102:1, 1955.

[15] Straus, W., Isolation and Biochemical Properties of Droplets from the Cells of Rat Kidney. J. Biol. Chem., 207:745, 1954.

When a portion of a proximal convolution from a normal rat kidney was examined in Locke's solution, unfixed and presumably still living, the mitochondrial rodlets could be seen dimly. The addition of 1/20,000 Janus Green to the solution sharply brought out their contours by supra-vital staining (Figure 1). When a similar preparation from a rat which had received and which was excreting egg white was examined, the rodlets had disappeared and the cells were filled with large droplets. By fortunate mischance I happened to crush the cover glass in changing to oil immersion and on examining the remains of the specimen noticed that the droplets had been forced from the disrupted tubule and were now scattered through the surrounding Locke's solution where they floated about quite intact (Figure 2). It was evident at once that they could not be droplets of protein as had been supposed by earlier investigators or they would have dissolved The assumption of an admixture of some substance insoluble in water to account for their insolutility suggested the presence of a lipid; the mitochondria, rich in this material, had disintegrated and this seemed a possible source. Supravital staining with Janus Green showed in fact that the droplets reacted positively and the conclusion therefore seemed reasonable that they were a mixture of absorbed egg white and mitochondrial substance. A hint as to the metabolic significance of this combination appeared when the green dye within the droplets was promptly reduced to its red form, an observation which seemed to suggest that some catalytic or enzymatic activity had been transferred with the mitochondrial component to the protein droplet.

These observations[8] were, you might say, the seminal experiment from which all our future investigations grew and, as you see, it contains in a crude form all that subsequently was more specifically demonstrated. What followed was a more exact testing of the hypothesis that the droplets are a mixture of absorbed protein and mitochondrial substances and their enzymes by various appropriate techniques: A. Biochemical techniques, in the ordinary sense of the word, in which the droplets were spun out of homogenized suspensions of cells and their phospholipid and other constituents quantitatively determined[15]. The results showed that they did contain all the materials of the mitochondria as well as the absorbed protein. B. Histochemically, the droplets reacted in a way that indicated a mixture of mitochondrial stuff and the absorbed protein[10]. C. Immunologically, they reacted specifically, for example, to egg white precipitins six times more strongly than did any other cell constituent[14]. D. Enzymatic determinations[12, 13, 15] (many of which were done by our chairman, Dr. Kretchmer) revealed that their activities resembled but were not identical to the activities that are found in mitochondria.

We can pass now from considering the nature or constituents of the droplets to some of the situations that determine their formation. When do droplets form, and when do they not form in the presence of proteinuria? Perhaps I should start with what may seem like an unnecessary comment, namely that for intracellular protein absorption droplets to form in the renal cells, protein must enter the cell. This may seem obvious but the statement is necessary because some pathologists have been much puzzled how in various toxic nephropathies, natural or induced, such as a sublimate kidney, the urine can "boil solid" although there are no droplets in the renal cells. As we will see, there are many reasons why droplets do not form in renal cells. The simplest of these certainly is that a seriously damaged tubule cell absorbs little or no water, sugar, protein or anything else. The absence of droplets is not surprising, therefore, in a badly damaged kidney from which there is a proteinuria.

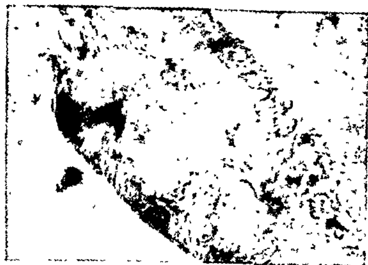


Fig. 1: Supravital staining by Janus Green in Locke's solution of the mitochondrial rodlets in an unfixed portion of intact proximal convolution of a normal rat.



Fig. 2 Similar preparation from a rat which had been injected with egg white and which was excreting this protein in the urine. *The mitochondrial rodlets have disappeared and the tubule is* filled with large droplets which stain in varying intensity with Janus Green. The tubule has been crushed and free droplets persist, floating in the suspending Locke's solution. They cannot therefore be droplets of simple protein.

One can demonstrate, as the next few statements will show, I think, that there are two cellular mechanisms concerned in the absorption of protein, one which may be considered physiological and the other accessory or pathological. Perhaps "mechanism" is too definitive a word. What I should say perhaps is that protein can be observed to be absorbed in two different manners and leave it as an inference that there are two different mechanisms. In both, I might add, the mitochondria play an essential part.

For instance, if a filterable protein, egg white or plasma protein, is introduced by injection directly into the blood stream of a rat it is absorbed by the epithelium of the proximal convolution and can be demonstrated [9, 16, 17] diffusely dispersed in a very short period of time, presumably in what one might call a molecular form throughout the cytoplasm of the cells. There are no droplets to be seen. If the sacrifice of the animal is postponed for an hour or so, one sees nothing; the protein has disappeared. It is quite definite therefore that in this molecular form of absorption, which is concerned with the immediate handling of small amounts of protein, there is no formation of droplets and the absorbed protein is disposed of in some way or other in a relatively short length of time. As we shall see later, this is apparently the manner in which the tubules absorb the small amounts of plasma protein which pass through the glomerular membrane as a continuing physiological process.

On the other hand, if the same protein, let us say egg white because it is a convenient one to demonstrate, is introduced into the peritoneal cavity in large amount it is gradually absorbed and excreted over a day or two. Under these circumstances egg white protein absorption droplets appear in great numbers in the renal cells. The great majority of these droplets disappear in a few days; some, however, may persist for quite lengthy periods, evidently, therefore, there is some difficulty in the disposal of egg white In other words, the cells have absorbed the protein and ultimately dispose of it, but in this case with some difficulty and droplet formation whereas in the first case disposal was prompt and by a simpler mechanism without droplet formation.

Examining this situation, it can be demonstrated that the dosage required to produce droplets varies with different proteins; large amounts and repeated intraperitoneal injections over a considerable period is required with homologous plasma proteins; a single injection of smaller amounts of a foreign protein is sufficient. For example, in rats it requires many repeated intraperitoneal injections of large amounts of rat serum to produce droplets, considerably less if one uses bovine albumin, and as I have said, a single injection of egg white suffices.

It can be shown by in vitro experiment [9] that the digestibility of the absorbed protein by the cell varies inversely with the facility with which droplets are formed. For instance, intracellular accumulations of plasma proteins, which must be injected in large amount to cause droplet formation, are digested in 3 hours by the renal cells

[16] Coons, A. and Kaplan, M. H., Localization of Antigen in Tissue Cells. II. Improvements in a Method for the Detection of Antigen by Means of Fluorescent Antibody. J. Exp Med., 91:1, 1950.

[17] Mayersbach, H. and Pearse, A. G. E., The Metabolism of Fluorescein-labelled and Unlabelled Egg White in the Renal Tubules of the Mouse. Brit. J. Exp. Path., 37:81, 1956.

on incubation at 37 degrees. On the other hand, it is a matter of days before intracellular accumulations of egg white are digested.

It might be concluded, therefore, that droplet formation occurs when the capacity of the normal molecular mechanisms of disposal is exceeded and absorbed protein accumulates and combines with mitochondrial substances in grossly visible form. Droplet formation is therefore evidence of a saturation of the normal or physiological *mechanisms of protein disposal in the cell.*

Saturation of disposal *mechanisms and droplet formation* are determined not only by the amount of absorbed protein and by its digestibility, but also by the catabolic capability of the renal cells. This latter feature may vary, *especially under adverse* conditions. Recent unreported experiments[18] demonstrate certain situations where droplet formation is the result of depression in the ability of the cell to dispose of a protein which under normal circumstances is readily digested.

Varying degrees of cell damage produce quite opposite results, either favoring or precluding droplet formation. As I have stated, if the cell damage is extreme, as in sublimate poisoning, no protein is absorbed and no droplets form. If, however, cellular function is only moderately disturbed, protein may be taken up by the renal cells but *disposal of it fails; accumulation occurs and droplets form. These relations have been* demonstrated in two sorts of experimental cell insufficiency.

Ischemia arising from clamping the renal artery produces in the kidney wide variations in cellular damage, from slight and temporary alterations to frank cell necrosis. Proteinuria resulting from glomerular damage accompanies the tubular lesion. In such kidneys droplets are observed in the better preserved cells; they have all the histochemical characteristics of those that follow the injection of large amounts of plasma proteins into normal animals. The additional injection of plasma protein into such animals *results in a filling of the entire proximal convolution with great* numbers of droplets. These results would seem to demonstrate that a depression of *mechanisms of enzymatic* disposal by anoxic damage may result in an accumulation of protein and droplet formation under circumstances where, if the normal disposal process had prevailed, no droplets would have formed.

Another form of tubular insufficiency can be produced by removing surgically 3/4 of the kidney substance. Platt[19] has studied this form of experimental chronic renal insufficiency and pointed out the similarity to what obtains in chronic glomerular nephritis where *the end result of the pathological alteration* is a *kidney* with a few nephrons on which the full load of renal activity falls. It is under such conditions that *the nephrotic syndrome* with "hyaline droplets" in the proximal convolutions is observed

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[18] Oliver, J., MacDowell, M. and Lee, Y.C., Cellular Mechanisms of Protein Metabolism in the Nephron. VII. The Characteristics and Significance of the Protein Absorption Droplets (Hyaline Droplets) of Human Renal Disease and Their Relation to the Physiological and Pathological *Absorption and Disposal of Plasma* Proteins, in press.

[19] Platt, R., *The Lumelian Lectures* Structural and Functional Adaptation in Renal Failure. Brit. Med. J., 1:1312, 1952.

in man. We have repeated these experiments and found that in the remaining remnant droplets form and that these can be greatly increased by injection of rat plasma proteins[18].

These experimental demonstrations that a reduction in the efficiency of the cellular mechanisms of protein disposal may result in the accumulation of absorbed plasma proteins in droplet form may be applied to the renal lesions of human disease where hyaline droplet formation is a not uncommon occurrence. I use the double negative because hyaline droplets are not so common, at least as I define them, as one might be led to believe by some other descriptions. The reason for this will become apparent in a moment. I have recently had an opportunity to observe these relations in a human disease under conditions which resemble very closely the procedure of the animal experiments[18, 20].

In the hypotensive phase of Epidemic Hemorrhagic Fever, very large amounts of concentrated human serum albumin were repeatedly given to support the circulation, anywhere up to 20 units (a unit being 25 grams in 100 cc.) were given in 48 hours and that is really a very large amount of homologous albumin. In the kidneys of those who died, hyaline droplets were found in numbers exceeding anything observed in our previous experiments. The entire proximal convolution of every nephron was crowded from glomerulus to terminal tip with huge droplets that had all the distinctive histochemical reactions of the experimental protein droplets[18, 20].

In comparing these findings in the human disease to those of the experiments, the condition of the kidney in EHF must be considered, it can be summarily described as a modified form of ischemic renal failure with varying degree of proximal tubular damage[21]. It might be recalled in passing that droplet formation has not been described as a striking feature of the ischemic lesion of acute renal failure, except in those cases where hemoglobinuria occurs, nor has treatment in the past included the injection of such great amounts of serum albumin.

As in the experiments, droplets formed in the human disease only after the injection of a considerable amount of the homologous plasma protein[18]. in a series of 39 cases, 25 individuals received less than 5 u. in 48 hours; droplets were not present in 17 cases. In 5 instances, however, they were present. These exceptions, I would suggest, may be explained by the complicating factor of reduced cellular catabolic activity which followed a renal ischemia similar to that produced in the clamped kidney. The same is true of one case which received no albumin, yet showed droplets.

Out of 14 cases who received more than 5 and up to 20 u., all but 5 showed droplets in great numbers. Of the 5 negative exceptions, one case was moribund during the administration of albumin which occurred during his last hours and at autopsy frank necrosis had occurred in the great majority of the proximal convolutions. Two cases received the protein on the 15th and 18th days of the disease for terminal shock, the epithelium of their proximal convolution had been replaced by atypical regenerated

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[20] Oliver, J. and MacDowell, M. C. The Renal Lesion in Epidemic Hemorrhagic Fever J. Clin. Invest., in press.

epithelium[20] and experiment has shown[8] that such cells do not absorb either vital stain or protein; hence no droplets were to be expected. The other two cases had survived the earlier injection a longer period than experiment has shown[9] to be required for the complete disposal and disappearance of droplets of homologous protein.

The irregularities in droplet formation observed in the human disease therefore "prove" the rules established in the experiments in the original sense of the word by "testing" their validity under various conditions of abnormal renal activity.

In general then, the human experiment of injecting serum albumin follows what one observes in the animal; if *very large amounts, over 5 units in 24 hours,* are injected, droplets form from a saturation of the disposal mechanisms unless cellular damage has been so severe as to prevent absorption of the protein; droplets may form, however, below this threshold if the digestive capabilities of the cells have been reduced by the renal lesion. In any one case of EHF doubtless the droplets seen have arisen by both circumstances as the lesions in the tubules vary greatly from nephron to nephron, as in all examples of acute ischemic renal damage.

There are other forms of renal disease in which hyaline droplets occur, but never to the degree that I have described in those individuals with EHF who were saturated *with serum albumin. At best, a few coils of the proximal convolution are seen lying* about each glomerulus rather than a filling of every cross-section in the cortex and outer medulla. The list of these diseases runs as follows: chronic glomerular nephritis, renal amyloidosis, conditions associated with hyperglobulinemia and Bence-Jones proteinuria, lupus erythematosus, malignant hypertension and subacute bacterial endocarditis. In all of these conditions alterations of some sort are electrophoretically demonstrable in the plasma proteins; we do not know the exact nature of this "plasma abnormality", but judging from our experiment in which it was shown that proteins which readily form droplets are resistant to intracellular digestion in vitro[9], it well may be that the renal cells are able to distinguish "abnormal proteins" more accurately than *the biochemists; this is certainly true in immunological procedures. It would seem* therefore reasonable to accept Randerath's original explanation[6] that in these renal diseases "abnormality" of the plasma proteins is a factor in droplet formation.

At this point I must digress for a moment to briefly mention a complication which arises in the course of the story I am telling of the "hyaline droplets". Although it has been assumed by many pathologists from the simple similarities of morphologic appearance and staining reaction that the hyaline droplets of these diseases *are analogues to* the experimental protein droplets, a lesser and, I believe, ever decreasing number of pathologists have vigorously objected to this conclusion since the more specific evidence I *have mentioned* has been accumulated. They insist, with no evidence with which I am familiar, that the droplets are "degenerative" in nature and are simple mitochondria swollen by "osmotic" forces[21]. According to this interpretation "hyaline droplets" can bear no relation to the absorption of protein.

It has long been known, ever since the fact that mitochondria existed, that the rodlets in renal cells may disintegrate and form objects which are at times called

[21] Allen, A. C., Clinicopathological Meaning of the Nephrotic Syndrome. Amer. J. Med., 18:277, 1955.

"granules"; they certainly are in fact "droplets" of some sort. I have published illustrations of these phenomena, that is, the transformation of mitochondrial filaments into droplets in living renal cells as they appear by phase microscopy[8].

Whether the hyaline droplets of our experiments, and by inference, those of human disease are so derived is a matter that can be settled with greater ease by experiment than by argument. I have described earlier the histochemical characteristics of the protein absorption droplets[10]. Although the staining of mitochondrial remnants (droplets) and hyaline droplets is in some ways similar, their reaction to histochemical procedures is quite different[18]. The differences derive from the fact that the protein absorption droplets contain a considerable concentration of absorbed protein, in certain instances egg white or hemoglobin, which is obviously not present in the simple mitochondrial granule of the normal animal. The latter can be produced either in vitro by placing bits of kidney in hypotonic saline or in vivo, as shown by Funk-Brentano[22], by hypertonic glucose infusions. The renal cells in both cases contain remnants of the mitochondrial rodlets which appear as granules or droplets. Similar granular-droplet disintegrations of the rodlets result from autolytic changes in the renal epithelium and from the action of strong nephrotoxic poisons, such as sublimate. All these granule-droplets of mitochondrial disintegration stain deeply, as do protein absorption droplets, with iron hematoxylin, probably because of the common phospholipid content which we have demonstrated, and both are eosinophilic. They, like the original mitochontrial rodlets from which they arise, are negative, however, to all the other procedures to which protein absorption droplets are strongly positive, namely, the Gram stain, the PAS reaction, and various histochemical procedures which indicate high concentration of protein. To all of these procedures the protein of the plasma in the renal blood vessels, that filtered into the tubule lumen and that concentrated in the hyaline droplets in the cells are strongly positive both in the experiments and in the human diseases previously mentioned[18].

It seems to me certain therefore that the droplets following injection of protein into animals and man and those occurring in renal disease are not simple swollen mitochondria, they are rather the pathological aspect of the absorption of protein from the tubule fluid and of its disposal by the renal cells. Droplets appear after saturation of the normal capacity of the renal cells to dispose of the absorbed protein which under physiological conditions filters through the glomerular membrane in low concentration, say around 20 mg. per cent. This may come about as a result of (1): the amount of protein absorbed; (2): its nature, i.e., its digestibility, or (3). from defects in the normal disposal mechanism that have resulted from cellular injury. The last two in conjunction with increased filtration of protein through the damaged glomeruli are common causes of hyaline droplet formation in renal disease, the first was illustrated in the administration of plasma protein in EHF.

After devoting so much talk to droplets, it probably will come as anti-climactic to state that I do not consider them the most important phenomena in the handling of protein by the nephron. Proteinuria and tubular resorption of protein may occur without

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[22] Funk-Brentano, J-L., Contribution a l'Etude du Mechanisms Physiologique de L'Anuria au Cours des Nephropathies Aigues. Imprimerie Cario, Paris, 1953.

droplet formation. I wish to emphasize that droplet formation is therefore not necessarily evidence of increased protein absorption and disposal, as is sometimes implied, but of difficulties and disturbances in these processes. Nor, conversely, does their absence indicate that absorption and disposal of protein are not active.

We shall hear, I hope, some of the recent evidences that indicate the importance of the nephrons in the continuing and physiological modification of the plasma proteins that occurs as they are filtered through the glomerular membrane and reabsorbed through the tubule wall. Simple arithmetic shows the magnitude of these processes. It is my hope that "hyaline droplets", so long a very minor if curious paragraph in the chapter of renal pathology, have proven to have had at least heuristic value in raising questions on more important matters, namely, the part played by the nephrons in the metabolism of the plasma proteins both in health and disease.

CHAIRMAN KRETCHMER: I would like to ask Dr. Oliver if he would comment on something that has been perplexing me. In most textbooks the term "athrocytosis" is used to describe protein absorption by the tubule. This indicates that the cells are engulfing bits of protein by some form of ameboid or pseudopodal type of action. The type of mechanism you have described implies something more dynamic. I wonder if you could comment on the correctness of the use of the term "athrocytosis".

DR. OLIVER: I have no information as to how the protein gets into the cell and I don't think that the idea of engulfing, pinocytosis, is necessarily part of the original concept of athrocytosis. In fact, I don't know what the current concept of athrocytosis really is. It is a very vague term, and I think it had been, not deliberately but subconsciously, used because its vagueness is an easy brush-off of a lot of difficulties; to say, for example, that protein is not absorbed, it is "athrocytosed". Now that we know more of how the droplets form, I prefer to use the simpler word and say the cells absorb protein. As to how the protein gets through the tubular membrane I have no evidence. One could imagine the brush border surrounding and engulfing molecules; electron microscopy shows deep channels extending into the depths of the cell which might favor such a pinocytosis; maybe they are pores. In any case the proteins pass through the glomerular membrane, so it is not surprising that they enter the tubule cells through a membrane.

CHAIRMAN KRETCHMER: I believe that Dr. Novikoff has done some work with new histochemical techniques for staining droplets. Also, Dr. Novikoff might discuss the problem of mitochondrial dissolution.

DR. NOVIKOFF: We are studying the droplets in the proximal tubule cells of the normal rat kidney, concentrating on enzymatic properties and electron microscopy.

With Dr. Holt, we have shown that these droplets retain their high esterase activity (revealed by Holt's indoxyl staining method) following short fixation in buffered osmium tetroxide (J. Biophysical and Biochemical Cytology, in press). Our first attempts to retain the indigo color through the methacrylate embedding required for electron microscopy have failed, perhaps due to the chemical catalyst employed in the polymerizing process.

We are particularly interested in studying the changes in the fine structure and enzymatic properties of mitochondria and of the "large granules" described by Rhodin in animals of different ages. This may tell us how different "droplets" of the tubule cell are inter-related.

DR. OLIVER: To get protein absorption droplets you have to have protein absorbed. *There are other sorts of droplets, however. Those you describe are certainly* not droplets of absorbed egg white, are they?

DR. NOVIKOFF: No.

DR. OLIVER: In the droplets from the renal cells which we got out of suspensions we could show immunologically the egg white in a concentration six times that of any other part of the cell[14]. In some other types of protein absorption droplets we did not need to go to such refinement. In the case of hemoglobin protein absorption droplets, they were brown. So the droplets we described were hemoglobin or egg white absorption droplets and certainly the droplets you show aren't either. We cannot a priori rule out hemoglobin perhaps because that is available in the animal's economy, but not egg white.

DR. BARNETT. Dr. Oliver, how would you describe the appearance of the kidneys of patients with nephrosis? You talked about a variety of diseases in which droplets are commonly found, what is your opinion of their frequency or magnitude in the kidneys of children with nephrosis?

DR. OLIVER: I have not seen many examples of large, plump droplets in the very few cases that I have examined of the so-called "nephrotic kidney" in children. I would think that it is the exceptional kidney that would look anything like an egg white kidney or a myeloma kidney or a kidney from the nephrotic episode of glomerular nephritis that is filled up with huge, solid-looking droplets, some almost the size of nuclei and which by histochemical reaction would show a positive PAS, Gram stain and protein reaction. You are covering such a broad field when you say "children with nephrosis" I would not expect any consistent finding. And in any case, as I said, much protein can be absorbed by the tubule cells in molecular form without droplet formation. It is only when there are difficulties of intracellular disposal of the absorbed protein that droplet formation is to be expected.

DR. KURT LANGE: I would like to ask Dr. Oliver whether he has any idea about the functional capacity of such tubules which have droplets in them. For the last few years we have batted around the idea that if the functional capacity of these tubules has gone down, isn't the nephrotic syndrome the result of leakage of large amounts of protein through the glomeruli and this protein damages the tubules. Then we have a sort of acute renal failure in a milder form due to the protein leakage which has damaged the tubules secondarily. Do we know anything about the functional capacity of these tubules?

DR. OLIVER: I am sure we know nothing. In fact, Dr. Josephson and I have been struggling with this problem, that is, with an experiment where proximal convolutions have been filled with droplets, doing PAH excretions, glucose Tm's and what not, to see

if they have been disturbed. Unfortunately, for technical reasons we have not gotten very far but we are still trying. The difficulty is that rabbits on which clearances are relatively simple to perform, are very poor animals in which to produce droplets, at least with egg white. Rabbits don't like eggs in any form, either the white or the yolk, as we know from experimental atherosclerosis. If you inject large amounts of egg white intraperitoneally it is not absorbed and when you do get droplets they are not the fine, large objects that one observes in a rat which absorbs the egg white from his peritoneal cavity very well. Of course clearances on the rat are difficult, so that the way I would like to do it is the way Dr. Josephson would like to avoid and vice versa. I do think it would be very unfortunate in testing this idea if experimental animals were used on which clearances were well done but whose cells were not thoroughly filled with droplets. What we need is to start with proximal tubules that are like the rat egg white experiment, with the cells completely filled so we can look at them and see just how many nephrons are filled and to what degree. This can vary from a very small part of a convolution to its entire length. If we can get this accurate information and clearances I think probably we will be able to answer this question.

There has been, of course, a lot of assumption in biological experiments that you can block the function of cells by just filling them up with something you can see. That may or may not be true. For instance, in the cat kidney, the cells of the proximal convolution are so filled with fat in a good, well nourished adult that you can hardly see any protoplasm in the cells of his proximal convolution. They look like huge fat globules and one might be tempted to say that these cells could not transport or absorb or secrete the way cells with a proper amount of cytoplasm do; yet cats don't have any difficulty that I know of in using their proximal convolutions.

DR. JOSEPHSON: I agree with Dr. Oliver that it would be very interesting to know if the droplet formation in some way influences other functions of the tubular epithelium, for instance, PAH excretion, glucose absorption, etc. As Oliver mentioned, we had technical difficulties. I wonder if it would not be of interest to examine how other species than these mentioned here behave when we give them egg white, because it might be of interest to compare the droplet formation with -- as Dr. Oliver points out -- the way in which the animal likes egg, and secondly, would it not be of interest also to study the formation of droplets in animals in which the tubular function has been changed by experimental measures? In rats where you can produce beautiful droplet formation, I don't know in which way by which mechanism protein is absorbed. I don't know if, for instance, benamid has any influence on the reabsorption of protein. If it has, it would be of particular interest to see if droplets appear in an animal treated with benamid or with an acetate according to Taggart's work or other measures influencing the tubular function.

DR. OLIVER: Has no one filled the proximal cells in kidney slices with vital stain? That would seem a very simple thing to do with Trypan Blue and other dyes and see if "transport" is altered.

DR. LAUSON: This ties in with the unresolved problem of why some cases of "pure" nephrosis in children are associated with enlargement of the kidney and with hyperfunction. I would not be surprised, if you could carry out the experiments you talk about, that you would find hyperfunction, i.e., increased filtration rate, blood flow and Tm for p-aminohippurate and glucose.

DR. OLIVER: I would not either. At least I certainly don't assume that there of necessity would be a suppression of function.

DR. LAUSON: I think this experiment ought to be done in the dog.

DR. BARNETT: Conversely, as long as one is guessing, the absence of consistent appearance of droplets in nephrosis may be a manifestation of hyperfunction.

DR. LANGE: Not necessarily. It may be a latent stage when we get the kidneys and these tubules are damaged and do not show absorption.

DR. BARNETT: There are probably innumerable guesses.

DR. OLIVER: I have a watertight argument about the effect of damage on the cell and droplet formation. If droplets form, I say that the damage was only sufficient to depress the disposal mechanism of the cell, if droplets don't form, that the cell was so badly damaged that it did not absorb protein. If this seems to be having it both ways, I might add that experimentally one can demonstrate this difference between the response to slight and to severe damage.

CHAIRMAN KRETCHMER: Dr. Brun, have you had an opportunity to examine biopsy material from children with nephrosis whose renal function is normal but who still have proteinuria?

DR. CLAUS U. BRUN: Well, our technique has involved fixation in alcohol. I don't know if Dr. Oliver will accept that.

DR. OLIVER: There will be no protein absorption droplets in such material.

DR. BRUN: We have seen something which looked a little funny. It was the holes where the droplets were. This was just in the kidneys where the PAH secretion was abnormal and the TM was normal, whereas in later stages we did not see it.

DR. OLIVER: One can take tissue from a kidney of a rat and fix it in say, Carnoy's fluid, or any fixative that contains a lipid solvent and also fix it in bichromate solution, the bichromate tissue will be full of droplets and not a single droplet will be seen in the lipid solvent fixed material. This is because a considerable part of the protein absorption droplet is phospholipid derived from its mitochondrial component.

DR. BRUN: Would "holes" be accepted as a sort of evidence of droplets having been there? If so, then I can answer yes to Dr. Kretchmer's question.

DR. OLIVER: It might be the result of removal of protein absorption droplets but it might be something else, as well. In the experimental tissues there are no holes left where droplets may have been, the droplets simply vanish without leaving any trace. I think we are involved again in the difficulty that there are "droplets and droplets" One has to be able to define them accurately as protein absorption droplets.

CHAIRMAN KRETCHMER: As usual, the morphologist has answered some questions but also has proposed a group of new and perplexing questions to the clinician and the physiologist. The advantage of the morphology as presented by Dr. Oliver is that one can examine the nephron in regard to the transport of protein in a non-statistical manner. This is in contrast to the methods of clearance studies which utilize the statistical approach. In order to present this aspect I would like to call on Dr. Wallace McCrory.

B. Studies of Tubular Reabsorption of Proteins in Humans

DR. McCRORY: The studies I am going to report were done in the main by Dr. Duncan Macaulay working in our laboratory during the last year. The aim of these studies was to determine whether significant renal-tubular resorption of protein can be demonstrated to occur in children with proteinuria due to nephrosis. The occurrence of significant proteinuria is now generally accepted as indicating increased glomerular permeability to serum protein, since tubular excretion of protein has not yet been demonstrated to occur. The question of interest is whether patients with proteinuria have a significant degree of tubular resorption of the protein being filtered through the glomerulus. A recent paper [23] reports suggestive evidence for significant resorption of protein in a group of patients with nephrosis. Another group of workers [24] have interpreted data obtained under similar conditions as suggestive evidence that there is relatively little tubular resorption of protein in patients with proteinuria of nephrotic origin. The method of study is indirect and makes use of the observation that for substances filtered through the glomerulus and excreted in the urine a relationship between the load (amount filtered) and excretion can be demonstrated. The fact that albumin excretion increases following elevation of plasma albumin induced by albumin infusion in patients with proteinuria is now well recognized. In the case of a substance freely filtered through the glomerulus and neither reabsorbed nor excreted by the tubule (Inulin) the regression of excretion (UV) on load (serum conc. x G.F.R.) is linear and the line describing the relationship will intersect the abscissa and ordinate at the origin. However, if tubular resorption occurs the regression line will intercept the abscissa above the threshold value of 0 and the projection of the line on the ordinate would give a negative intercept. This relationship has been used to demonstrate the occurrence of tubular resorption of glucose and phosphate. In the case of these two substances it has been assumed that a limiting rate of tubular resorption exists. The relationship of excretion (UV) to load (S. conc. x G.F.R.) when the filtered load exceeds a constant maximal rate of tubular resorption would be linear. The intercept of the regression line in this circumstance would be a measure of the magnitude of tubular resorption. Since protein is obviously not freely filtered by the glomerulus another variable exists in applying this case to the study of tubular resorption of protein and that is the degree of permeability of the glomerulus to protein. However, the slope of the regression line will provide a measure of the permeability of the glomerulus to protein. The permeability would also have to be a constant in order to obtain a straight line. Thus it would appear that this indirect method could provide useful information if one finds a linear regression of excretion on load. The equation for this function would be excretion ($U_{alb}V$) = K x load - tubular resorption. K would be the slope of the

[23] Hardwicke, J. and Squire, J. R., Clin. Science, 14:509, 1955.
[24] Chinard, F. P., Lauson, H. D., Eder, H. A., Greif, R. L., and Hiller, A., J. Clin. Invest., 33:621, 1954.

line and would measure glomerular permeability. A negative intercept on the ordinate would indicate the occurrence of and magnitude of tubular resorption of protein. This is the principle of the method Hardwicke and Squire have used to provide evidence of tubular resorption of protein.

DR. HOWARD EDER: It should be noted that tubular reabsorption of certain substances occurs without there being a limiting rate. Urea is an example of this. When the excretion of urea is plotted against the plasma concentration a straight line which can be extrapolated to the origin is obtained. Thus the finding of a similar curve for albumin (as we did) does not exclude tubular reabsorption. It only excludes the presence of a limiting rate, Tm.

DR. McCRORY: If proportional rather than a limiting rate of tubular resorption of protein occurs, as Dr. Eder states, the regression line would pass through the zero point. In any case, the hypothesis is suitable for testing and we can now go on to our data. The method of measuring the protein in plasma and urine requires some comment first. We have done considerable work with estimations of protein clearances in children with nephrosis and have obtained ample evidence that proteinuria in nephrosis appears to reflect molecular sieving of proteins in relation to molecular size and undoubtedly other physical characteristics It does not appear to represent the simple escape of serum protein into urine. Our evidence for this lies in the values obtained for clearances of the separate protein fractions. The albumin clearance is highest, the alpha-1-globulin next and the gamma next, while the alpha-2-globulin and beta-globulin clearances are lowest ($C_{\alpha\text{-}2\ glob}/C_{alb}$ = 0.01 - 0.10). This is in agreement with what one would expect with increased glomerular permeability to serum protein in view of the average molecular size of the proteins comprising the various fractions separable by electrophoresis. However, there are important differences between the proteins making up the various protein fractions in plasma and urine. The urinary alpha-2 and beta globulins, unlike the serum counterparts, contain little or no lipid, the urine alpha-1-globulin fraction contains considerably more muco-polysaccharide than the same fraction in plasma. We have attempted an estimation of the clearance of the alpha-1-glycoprotein (molecular wt. 45,000) but have been unsuccessful because of technical difficulties. We have estimated the iron-binding globulin clearances in two subjects (molecular wt. 90,000) and found it to be considerably higher than the estimated beta-globulin clearance in these subjects. In short, the measurement of clearances of proteins demands a more specific method of measurement than electrophoresis for any of the globulin fractions if the clearance data are to be considered as reliable measurements for interpretation of glomerular permeability or tubular resorption of protein. Accordingly we chose to study a single protein moiety, albumin, and used immuno-chemical methods for its estimation in serum and urine. The subjects were children with nephrosis. They received an infusion of 25 gm. of salt-poor human albumin and urine and plasma samples were then collected at two hour intervals for 8 hours, and then at 4-6 hour intervals. The data for each study included the 20 hour period following the infusion. In most instances a second infusion was given and similar samples were obtained. We have analyzed the data for each infusion separately. Glomerular filtration rate was estimated by measuring the endogenous creatinine clearance for each individual period. We had a total of seven observations on three children. Figure 3 represents the method of applying the data to observe whether evidence of

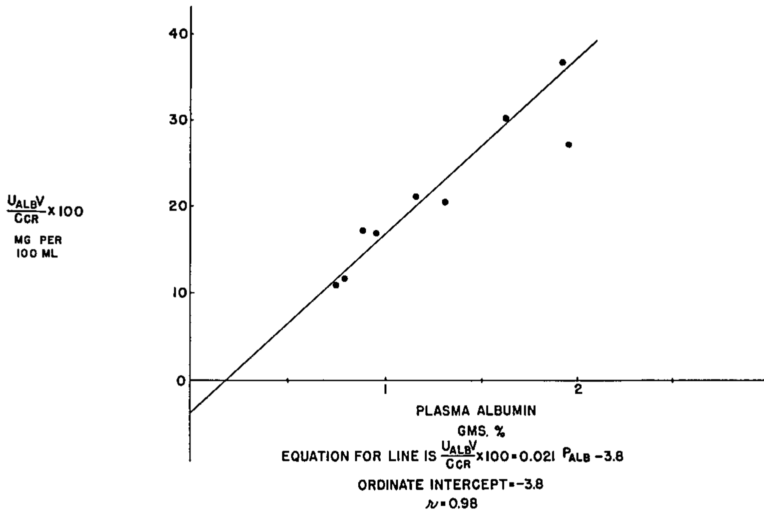


Fig. 3. Urinary albumin excretion in relation to changing level of serum albumin following the infusion of human albumin in a child with nephrosis.

tubular resorption of protein can be obtained by this method of study. The ordinate *represents urinary excretion* and was calculated as indicated by dividing the albumin excretion (mgm./min. ($U_{alb}V$)) by the creatinine clearance (Ccr). Multiplying this by 100 provides a measure of mgm. of albumin excreted per 100 cc. of glomerular filtrate. Thus the ordinate intercept would indicate mgm. of albumin excreted or resorbed (if negative intercept) per 100 cc. of filtrate. The abscissa represents load, presented here as gm.% of plasma albumin. The correlation coefficient for the linear equation was quite high (r = 0.98) in this instance. We felt five of the seven experiments had acceptable values for r (.98, .84, .78, .93, and .92) and the intercept of the regression lines were -3.8, -.9, -.8, -5.7, and -9.1. Because of the tedious problem of acquiring such data and our small number of samples we have turned to the data of the two groups cited above to enlarge our series. Four observations following a single infusion reported by Chinard, et al., were analyzed by us in a similar manner. The correlation coefficients for the linear equations were (.92, .98, .96, and .97) and the intercepts were -63, -54, -9.5, and +2.5. We found two observations in the report of Hardwicke and Squire that seemed to be suitable for calculation in terms of albumin only. These authors calculated total protein excretion and, as stated above, we have great hesitancy about accepting the electrophoretic measurements for this type of calculation. the correlation coefficients were .91 and .92 and the intercepts were -10 and -36. The one seemingly significant finding would appear to be that in 10 of the 11 instances the sign of the *intercept is negative. The meaning of the wide range of variation in values for the* magnitude of tubular resorption is unclear. The conditions in the three groups were different in one important aspect. Our subjects had minimal proteinuria in comparison to the other workers' subjects. A more important factor that deserves consideration is the question of the constancy of the glomerular permeability to albumin under the conditions of these observations. Chinard et al. observed a marked increase in $U_{alb}V/Ccr$ in relation to load when plasma albumin concentrations above 2.5 gm.% were observed. They attributed this increase in the slope of the regression curve to an increase in glomerular permeability to albumin related to acute expansion of plasma volume in response to albumin infusion. It is clear that any change in glomerular permeability to albumin occurring during these experiments would alter the slope of the line. If it increases, as albumin concentration rises, the slope would increase and the negative value of the intercept would also increase Thus the differences in value of the negative intercepts could reflect in part changes in glomerular permeability. When regression lines were calculated for excretion when data were restricted to periods when plasma albumin values were of 2 gm.% or less the magnitude of the intercept decreased considerably. While Chinard et al. felt that increased glomerular permeability was an important factor only when plasma albumin was most markedly elevated we believe this has not been proven and is an arbitrary assumption. If alterations in plasma volume are associated with an alteration of glomerular permeability to protein this may be a factor at all levels of serum albumin and the regression of $U_{alb}V$ on load would not be linear but a curve and it would approach the origin. This is in agreement with the trend of much of the data as we see it. A further factor that could contribute to a change in the slope would be an alteration in tubular resorption of protein during the period of observation. Methods currently at hand do not provide a means of separating these variables and we cannot solve this dilemma by this approach. Consequently, we can conclude only that the data are compatible with the existence of tubular resorption of albumin. The variation in values for tubular resorption

could be due either to changes in glomerular permeability to albumin or changes in tubular resorption during the period of study. We doubt that this approach provides a quantitative answer to this all-important question in nephrotic patients.

DR. METCOFF: Did you by chance look at some of the data that have accumulated from dextran infusions? In some of these studies plasma volume was acutely expanded and albumin clearances were measured. There was a striking change in albumin clearance with or without change in simultaneous glomerular filtration of water[25].

DR. McCRORY: This would be most interesting. As I stated before the negative value of the intercept is lower if one arbitrarily selects points below serum albumin concentrations of 2 gm.%. The one observation in which serum albumin was not elevated above 2 gm.% was one from the Chinard data and that intercept was the one positive intercept (+2.5) of the group. This is as close as we can come to an answer when albumin concentrations are low. Selecting the portion of the data that appear to give the best straight line seems to me to be too arbitrary. We don't know what would happen as we approach zero. This question is of great importance because we are faced with evidence of very puzzling differences in rates of tubular resorption of protein in these patients. Alterations in some of our "assumed" constants and technical errors would seem to warrant close scrutiny before accepting the data as quantitative, to our mind.

DR. COOKE: Would you comment on the changes on the intercept of other proteins as you infused albumin? This finding might contribute something to the separation of the question of increased permeability or changes in reabsorption.

DR. McCRORY: We did not attempt to correct for total protein excreted or estimate the effect of albumin infusions on globulin excretion. Such data should have a high degree of accuracy if they are to be useful in solving this problem. The quantity of the various globulin fractions in urine during albumin infusions make up a very small fraction of total protein. Small errors in individual fraction estimates would result in large mathematical errors in subsequent calculations. We do not feel that the methods are suitable. Certainly protein fraction clearances based on such methods have limited value except for the albumin which is similar in urine and plasma and can be measured quite accurately by electrophoresis.

Dr. BLAINEY: May I comment on that? My colleagues, Drs. Hardwicke and Squire[26] have done the same thing as Dr. McCrory with the difference that the protein clearances were done on the falling curves after albumin infusion. The same sort of values were obtained for the intercepts. Total protein was used rather than albumin for the reason mentioned by Dr. Cooke, namely that it was found that the globulin clearance rose with the increase in albumin excretion. It seemed likely that this might indicate some competitive reabsorption of different protein fractions by the tubules and it therefore seemed necessary to make allowances for the globulin clearances in calculating the intercepts.

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[25] James, J., Gordeilo, G. and Metcoff, J. Effects of infusion of hyperoncotic Dextran in children with the nephrotic syndrome. J. Clin. Invest. 33:1346, 1954.
[26] Hardwicke, J. and Squire, J. R., Clin. Sci., 14:509, 1955.

DR. McCRORY: If you assume that the globulin clearances have the same validity that albumin clearances do, your interpretation is reasonable. The serum concentrations of the globulin fractions are much higher than the urinary excretions while the reverse is true for albumin. The introduction of corrections for tubular resorption of globulins is certainly desirable but it appears to be a labor of love to me at this time. We assumed that globulin resorption constitutes an approximately constant amount of tubular resorption of protein during the period of study and consequently should not prevent the demonstration of albumin resorption. We have assumed that proteinuria is itself evidence that the existing limiting rates for tubular resorption of protein have been exceeded. If protein resorption is changing as serum albumin is increased our data do not suggest that this occurs in a reproducible manner (i.e., increased or decreased). Consequently, I don't see that this question, granting its importance, alters the interpretation of our data.

DR. BLAINEY: We have assumed that the permeability was not altering. If it is changing, your question is difficult to answer, and I am not sure whether you will get more albumin or more albumin plus globulin. The latter seems more likely.

DR. McCRORY: I don't see how it would have a great deal of import in this specific problem, though we are working here simply with one species and trying to demonstrate the magnitude of reabsorption of this species.

DR. BLAINEY: The reabsorption of that species is altered by the reabsorption of globulin. That is the whole point.

DR. McCRORY: I don't disagree with this. I would be very happy if we are willing to accept this as evidence that albumin is reabsorbed under these conditions.

DR. PHYLLIS A. BOTT: There is a little information available, at least in amphibia, on the effect of changing the protein concentration on the amount of protein that comes through, in other words, the per cent of glomerular permeability. Some experiments[27] that Dr. Richards and I performed back in the archaic period that Dr. Oliver spoke of, indicated that in perfused kidneys, varying the protein concentration of such things as egg albumin and lactoglobulin, which we could determine in glomerular filtrate did not seem to have any effect on the per cent filtered. However, in some experiments that I ran later with hemoglobin[28], I did seem to get some increase. I think hemoglobin is a rather special case however. I have said a number of times before that more experiments of this type should be done. Methods much better than those that we have used are available now.

DR. McCRORY: I don't want to leave the impression that I don't think the globulin clearances have anything to do with this. I am sure that they do. If, as Hardwicke and Squire reported, more globulin is excreted, your point then is if you are reabsorbing more globulin this might depress the albumin further and this would be a factor you would have to introduce into the calculation?

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[27] Bott, P. A. and Richards, A. N., J. Biol. Chem. 141:291, 1941.
[28] Bott, P. A., Fed. Proc. 8:186, 1949.

DR. BLAINEY: Yes.

DR. McCRORY: I am sure that this exists but I would think that one could still keep this as simple as possible. This is complicated enough working with just albumin. Where we have ended up then is that either reabsorption is not a constant or permeability is not a constant. But I think it is fair to conclude that it is interesting that all intercepts, except one, are negative.

DR. LAUSON: Dr. McCrory knows well that we have never discouraged the experiments he went on to do but that we are vigorously opposed to the idea of his trying to squeeze too much out of this type of data. So I feel that his last remarks are the ones that probably are still the most applicable; that is, either glomerular permeability changes or tubular reabsorption changes. However, I still believe everything we said in the paper by Chinard et al.[24].

In experiments on glomerular permeability I would like to see a protein used which is normally present in low concentrations in the plasma and which therefore comprises only a small fraction of the total colloid osmotic pressure of the plasma. Radioactive iodine-labelled albumin is not satisfactory. However, if good analytical methods for the iron-binding globulin are now available, this protein would be an excellent indicator of permeability. Its clearance is of the order of magnitude of that of albumin. A two- or three- or even ten-fold increase in its plasma concentration would have little effect on the colloid osmotic pressure, therefore, there would be little effect on plasma volume. All of the complicating effects of acute expansion of blood volume during the course of the observations, such as do occur after administration of albumin, would be avoided.

Concerning glomerular permeability to different proteins relative to permeability to water, one can think in terms of the simple model of the old pore hypothesis. Suppose there are 99 small pores in a unit of surface through which water can pass readily and one pore that is just large enough to let albumin go through. If now one additional pore is enlarged to permit passage of albumin, everything else being the same, the amount of albumin coming through would be about doubled whereas the increase in passage of water would be relatively slight, or at least less than doubled.

DR. OLIVER: May I ask a question? I am not clear as to just what is being said, which is not surprising because I have no working experience with these methods. Is there functional evidence by clearances under any conditions that indicates protein is reabsorbed by the tubules? If I understand it, no one has offered any other explanation for the appearance of a threshold.

CHAIRMAN KRETCHMER: Dr. Blainey, would you care to comment?

DR. BLAINEY: I don't think any other explanation has ever been put forward. It has always been assumed that alteration of the clearance of the threshold substance must imply tubular reabsorption.

DR. LAUSON: I must take serious objection to that statement. These slopes can equally be explained as acute changes in permeability, even allowing for some tubular reabsorption.

DR. OLIVER: But that is not my question. I am not speaking of the change in the slope of the curve and the resulting increase in the negative intercept. I can understand how this could be the result of increased glomerular filtration of protein. My question is, when the extrapolation of the curve cuts the x axis and does not end at zero, what else can it indicate besides a threshold of tubular reabsorption?

DR. LAUSON: Change of permeability. The abscissa which Dr. McCrory talked about is the plasma concentration of the protein in question and the ordinate is its excretion rate (or excretion rate/filtration rate of water). The plasma concentration is raised acutely by infusion of the protein, and urine and plasma specimens are collected during several hours as the plasma concentration falls. Commonly, the data fall along a more or less straight line which often extrapolates to a negative ordinate value for plasma concentration of zero. I doubt the validity of extrapolation in these acute experiments for the reason that the acute expansion of plasma volume may induce acute increases in glomerular permeability -- the very entity which is assumed to remain constant (see Chinard, et al.[24]). The extrapolation is based on analogy with the relationship between the amount of glucose filtered (GFR x plasma concentration) and excretion of glucose. However, in the case of the protein it is assumed that the amount filtered is equal to GFR x plasma concentration x a constant permeability factor. Unfortunately, it is very unlikely that the permeability factor is anything like constant in these acute experiments. Furthermore, it is only a small fraction of the permeability to water, usually less than two or three per cent. This means that for each unit volume of plasma water which is filtered, only two or three per cent of the contained albumin can accompany the water through the glomerular wall. If permeability increases during the observations the extrapolation method cannot be used to evaluate tubular reabsorption of the protein.

DR. OLIVER: I understand this, I think. In other words, large increases in the amount of albumin filtered obscure changes in reabsorption; but do they prove that no reabsorption is occurring?

DR. LAUSON: If the plasma albumin is acutely elevated by intravenous administration of albumin, the ratio of excretion/plasma concentration, that is, albumin clearance, increases. We attribute this to increase in permeability related in some way to the associated acute expansion of plasma volume. The extrapolated curve resulting from this has a negative intercept. We agree that on the basis of morphological observations there is probably some reabsorption of proteins from the tubular fluid.

DR. OLIVER: Then it comes down perhaps to the situation that although there is evidence that proteins are reabsorbed by the tubules, there are very obvious difficulties in measuring that absorption by clearances, particularly in the abnormal kidney of nephrosis.

DR. LAUSON: Yes.

DR. OLIVER: That clearances are difficult to interpret when the nephrons are abnormal does not, of course come as a surprise to me[29].

[29] Oliver, J., J. Exp. Med., 73:141, 1941.

CHAIRMAN KRETCHMER: I am glad that this section ended on a note of agreement to disagree. I think that we are running short of time so we have time for one more formal presentation.

We have examined by rather careful techniques the morphological status of the nephron and by equally as careful but more confusing techniques the statistical problem of clearances. Dr. Hughes has investigated the problem of protein metabolism in the kidney.

C. The Major Role of the Kidney in Catabolism of Serum Albumin

DR. W. L. HUGHES: I have been interested in plasma proteins for a long time but not from the point of view of kidney physiology. My present interest stems from the following considerations:

First, I do not think that plasma albumin is prepared just to be catabolized by the body -- it has some nobler function in life! And yet it is catabolized and catabolized very regularly and rapidly. Now where is this going on? I would further add that this cannot be a "wearing-out" process since serum albumin is much too stable. Serum albumin has a half-life of months at 50°C, which would extrapolate to years at body temperature. Therefore, the observed half-life of one month or less in man can hardly be a result of denaturative changes. Furthermore, the same albumin disappears from the rat some 20 times faster still.

Last spring it occurred to me that the kidney would be a very likely major site of degradation not only for serum albumin but also perhaps for other serum proteins. This would follow if one assumed that a small amount of albumin filtered through the glomeruli and was reabsorbed in the tubules by a digestive process. In other words one would hypothesize that the tubule does not know how to conserve intact protein but returns it to the blood stream as amino acids following digestion.

If one makes this assumption and further assumes that all of the breakdown of serum albumin occurs in the kidney, it is simple to estimate the concentration of serum albumin in the glomerular filtrate necessary to provide the observed rate of degradation. Thus, a body pool of 250 grams of albumin in a 60 kilogram man with a half-time of 20 days would disappear at the rate of 3.4% or 8 grams per day.

Now 8 grams in 180 liters of glomerular filtrate (a figure I have taken from Homer Smith) corresponds to only 44 milligrams per liter in the glomerular filtrate. This is too small an amount to detect by usual techniques and certainly could not have been measured by the methods used in the classic studies of Richards and Bott. Forty milligrams per liter represents only about one-tenth of one per cent filtration; that is 0.1 per cent of the level in plasma would appear in the glomerular filtrate.

A simple way to look for kidney degradation would be to study the effect of nephrectomy upon the rate of albumin disappearance. We first tested this with rats. Iodinated serum albumin (either bovine or human) was injected into a rat and its disappearance rate followed by blood sampling. After about 36 hours (about 1 1/2 half-lives) when the rate of disappearance had reached a steady exponential decay, both kidneys were

removed and the rate of disappearance followed for another 36 hours. The rate of disappearance now fell to about half that originally observed.

We are not very good physiologists. Our sampling techniques were not good. We were getting a continuously decreasing hematocrit which perhaps was due to the size of the samples we were taking; or perhaps something else was going on. However, a correction for the apparently increasing plasma volume would only serve to magnify the change observed. Nevertheless, quantitative data now seemed difficult to obtain since we were only measuring the plasma concentration and could not readily estimate changes either in plasma volume or in extra-vascular pools. Therefore it appeared better to measure the total content of albumin in the animal and so we turned to the study of mice.

Now our procedure was not one of serial samples but rather each point on the curve represented one mouse sacrificed and analyzed. First, we repeated by double nephrectomy our studies in the rat and again found a markedly decreased rate of catabolism. However, the best data I would like to show did not involve nephrectomy but merely ligation of the ureters. We went in through the belly of the mouse and clamped off both ureters with little gold clips (Dr. Stoner in our laboratory did this part of the experiment.) Then after allowing about three or four hours for the animal to recover from the operation, iodinated bovine serum albumin was injected and animals were sacrificed after various times. The amount of labeled albumin relative to its degradation products was measured in a scintillation counter on trichloracetic acid precipitates following homogenization essentially of the whole animal. The homogenization was carried out on the skinned carcass. Since the skin was difficult to homogenize, it was counted separately and all of its activity (approximately 20 per cent of the total) was assumed to be protein bound. As you will see in the accompanying figure, we simultaneously ran controls on both normal mice and on "shams" in which the operation was performed with the exception of clamping off the ureters. the belly was opened, the intestines lifted out, put back in, etc.

In Figure 4 you will notice that the data has been plotted semi-logarithmically because of the anticipated exponential decay and you will notice that the data does fall quite well on straight lines. The half-life of 30 hours observed for normals (solid circles) is close to what others have found for normal mice. The open circles represent the animals with ureters clamped and the triangles represent shams. To me the differences are quite striking! The rate of degradation in the animals with ureters ligated is less than one-third of that in the normals. In contrast, the shams seem to show an accelerated rate of disappearance making the difference even greater.

DR. METCOFF Do the open circles refer to both the clamped-off ureter mice and nephrectomized mice?

DR. HUGHES: They only refer to the mice with clamped-off ureters although a similar effect was obtained by nephrectomy. Here the kidneys were intact but certainly not normal. During the course of the experiment they showed progressive enlargement.

DR. COOKE: How long after the clamping were the injections done in this study?

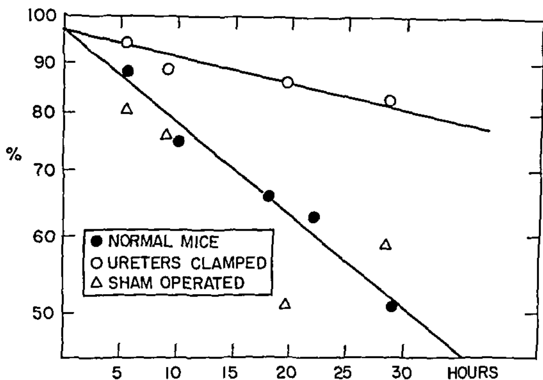


Fig. 4: Relative rates of protein degradation in mice following injection of I^{131} labeled bovine serum albumin into mice. Per cent of I^{131} precipitable by T.C.A. at indicated hours following injection.

DR. HUGHES: About four hours were allowed to elapse between clamping off the kidney and injecting the labeled albumin. We thought we would give the mice a little time to recover from the effects of anesthesia and surgery. Also I thought it might be important to fill up the kidney, pelvis, etc., with urine so as to stop all filtration before injecting the albumin. It seemed to me that otherwise after blocking off the ureter there might be a little initial degradation while flow down the tubule continued as the pelvis expanded from the pressure of urine.

DR. METCOFF: Did you ever clamp off the blood supply to the renal pedicle?

DR. HUGHES: We removed the kidneys completely.

DR. HOWARD GOODMAN: Do we understand you did it with nephrectomy as well as with ureters tied?

DR. HUGHES: Yes. The figure shows my best data but in all cases the general effect has been to halve the rate of degradation following removal of the kidneys in the rat, removal of the kidneys in the mouse, or tying off the ureters in the mouse.

CHAIRMAN KRETCHMER: Is the adrenal gland injured during this procedure?

DR. HUGHES: I don't see how it can be in the experiment where only the ureters were clamped. Dr. Stoner is very careful in his work. He just goes down to the ureter, barely lifts it out of the enveloping fat, puts around it a little gold clip, which he squeezes closed.

DR. COOKE: Did you take out one kidney by any chance and see if it went half way?

DR. HUGHES: No, we have not yet tried that experiment. I have some questions. I am disappointed because the rate of degradation did not fall to zero. It would be a much clearer experiment. Of course if the sham has really increased degradation elsewhere in the body, the results are more striking. I am fairly well convinced that the kidneys are a major site of disappearance for albumin. But where is the rest of the degradation occurring? Shall I try next removing both the kidneys and the liver to see whether I can decrease the rate of degradation to zero?

DR. MILTON RAPOPORT: Are you able to draw this conclusion? All you can validly say without getting too far out on a limb is that cessation of renal function is associated with decreased degradation of albumin. The effect could be in the kidney or be a general systemic effect of uremia.

DR. HUGHES: Uremia? Should not that be a progressive change due to accumulation of toxins? In this case should not the rate of degradation change progressively with time?

DR. RAPOPORT: Maybe I should not have said uremia. Maybe just the effects of nephrectomy.

DR. METCOFF: That is, failure to remove urine.

DR. RAPOPORT: Or incipient effects of loss of renal function.

DR. FOX: An even more serious objection, didn't Whipple show heterologous proteins are removed extremely rapidly?

DR. HUGHES: I don't believe this can be the explanation. The fate of heterologous protein varies with the protein used and I would state that to me another interesting fact about the catabolism of plasma albumin is the non-specificity of the reaction. Bovine, human, or rabbit albumin (iodine labeled) disappear equally as rapidly from the rabbit provided the labeling is properly done. I believe that the body cannot distinguish -- short of the immune mechanism -- between these various albumins. This further argues in favor of some mechanism such as the one I am proposing where the rate is regulated primarily by the diffusibility of the protein.

DR. FOX: People in the last few years have published data on labeled proteins for different species.

DR. HUGHES: You can have bad labeling. If the protein is denatured, the rate of degradation is greatly affected.

DR. LANGE: Dr. Rosenman showed last year the technique in which he reimplanted the ureter in the vena cava and the urine went back into the system again.

DR. HUGHES: This might make a very good control. I would picture what would happen, provided the kidney was operating normally: one should get the same rate of degradation as in the normal mouse but the degradation products should build up in the body.

DR. JOSEPHSON: It would be a good idea to use albumin from one mouse fed with radioactive carbon and inject that in another mouse or rat.

DR. HUGHES: I think, yes. I won't say that the homologous experiment should not be done. Certainly it should be done. However, I am sure my results cannot be explained on the basis of foreign protein.

DR. WHITE: What did your calculations show in terms of actual amount of serum albumin worked over by the kidney.

DR. HUGHES: About 8 grams per day in humans.

DR. WHITE: In terms of your initial calculation of 8 grams of albumin a day in the normal you then have an approximately three-fold increase in your half-life in the clamped-off animals?

DR. HUGHES: The calculations I gave were on humans. My experimental results are on mice.

DR. WHITE: I see. Have you done this in normal mice?

DR. HUGHES: No, I have not.

DR. WHITE: In terms of the half-life of albumin?

DR. HUGHES: No.

DR. WHITE: Then apropos of your other question, that you were surprised it did not fall more, I think it has already been fairly well covered by other comments. Certainly as a foreign protein this material is removed by many other tissues as well.

DR. HUGHES: This is where I disagree. Certainly distribution will depend upon the particular serum protein. In this case heterologous serum albumin behaves so much like the animal's own that it cannot be the explanation.

DR. ROSENMAN: Didn't you show that tissues will pick up serum albumin?

DR. WHITE: Yes.

DR. HUGHES: Dr. Gitlin has shown tissue picks up homologous serum albumin. Therefore I believe that heterologous serum albumin is going to the same places as normal albumin.

DR. WHITE: The point that has come up, Dr. Hughes, relates to your expression of disappointment, namely, did you expect that the entire degradation of albumin, which disappeared, occurred in the kidney?

DR. HUGHES: I had hoped it might.

DR. WHITE: It would make the matter a good deal simpler.

DR. HUGHES: Frankly, of course, I agree that alternate explanations are available. My results could be due to some peculiar physiological effect of nephrectomy as others have cited, perhaps some hormonal effect which is going around through the back door. Against this interpretation I would point out that to the best of my data this isn't a gradual effect but an immediate effect. If it were hormonal, it ought to be building up with time.

DR. LAUSON: How much of the disappearance in the normal animal is accounted for by the excretion of intact labeled albumin in the urine itself?

DR. HUGHES: I did not tell quite the whole story. During these experiments, the animals were without food or water. A clean piece of filter paper was put in the cage. The urine on the paper was collected and it was also precipitated with trichloracetic acid. About 10 per cent of the activity in the urine of mice is precipitable by trichloracetic acid and these corrections were included so that the graphs show only degradation.

DR. LAUSON. How much organically bound but non-protein radioactive iodine do you find in urine?

DR. HUGHES: Essentially none.

DR. LAUSON: The degradation products are simply excreted in the urine or have gone back into the body.

DR. HUGHES: There is a suggestion of intermediates in the degradation. It is presumed that the iodine is on a tyrosine group in the albumin molecule. In degradation presumably the protein is first hydrolyzed to the amino acid, monoaminotyrosine, which is subsequently deiodinated. There are thus two steps for complete degradation. I have a technique for separating iodide from the iodo-tyrosine: Iodide can be extracted into organic solvents with methyl mercury. In this way it appears that I can get essentially the same data analyzing for iodide as for non-precipitable iodine, since about 90 per cent of the non-protein activity is present as iodide.

DR. LAUSON: In the degradation of protein that you are assuming occurs in the kidney, does most of the iodine get reabsorbed and not put into the urine?

DR. HUGHES: In what case?

DR. LAUSON: Just from the difference between the two experiments that you described. Assuming all the albumin is picked up by the kidney, the difference between these two experiments is that all of the albumin is ultimately degraded in the kidney. The degradation products must be formed there and therefore must either go back into the blood or be excreted in the urine.

DR. HUGHES: Actually you know if the animal does not have a kidney then the iodide preferably goes into the stomach. There is a very high concentration of free iodide in the stomach, probably related to hydrochloric acid. I would say in the nephrectomized animal, perhaps up to half of the free iodide can be found in the stomach or gastrointestinal tract.

DR. WALTER HEYMANN: Like Dr. Rapoport, I believe that all one can say from these experiments is that in rapidly developing uremia, induced by nephrectomy or by clamping of the ureters, one obtains the described effect. One could, however, delay the development of uremia. After bilateral nephrectomy, one can keep rats alive two to four days by withholding food and water. With the help of varying procedures of vivo dialysis, like an artificial kidney, or peritoneal lavage, or the administration of large amounts of fluids, one can keep bilaterally nephrectomized dogs alive much longer. I wonder if one would still get the same effect under these conditions.

DR. HUGHES: That is a very good suggestion! Also what about using a chemical blocking of tubular reabsorption such as mercury poisoning? Is there any suitable agent for blocking reabsorption without otherwise damaging the glomeruli or the tubules so you could get normal glomerular filtration and still block reabsorption?

DR. ROSENMAN: Small doses of uranium might do the job. It might not leave you a normal glomerulus. Something like normal filtration should remain.

DR. SCHIFF: It would increase the protein in the tubule.

DR. COOKE: What was the assumed concentration in your glomerular filtrate in the human? I thought you said 20 mgs. per cent; therefore there would be 35 grams as I calculate it.

DR. EDER: I wonder about the figure of 35 grams per day for rate of degradation of albumin in the human. I would estimate the figure to be about 8 grams per day. I would prefer the lower figure since this would involve filtration and "reabsorption" of less protein. The figure of 8 grams per day is derived by using a T/2 of 20 days. The turnover time would be about 30 days. If we assume a plasma volume of 3,000 ml. with an albumin concentration of 4 gm. per 100 ml., the total circulating albumin will be 120 gms. per day. This would mean 4 gms. per day are degraded. Assuming that serum albumin constitutes half of the total body albumin pool and that extravascular albumin is in rapid equilibrium with plasma albumin, the figure of 4 gms. per day would be doubled.*

DR. GOODMAN: The ureter in the non-nephrectomized animals was clamped, is that correct?

DR. HUGHES: Yes.

DR. GOODMAN: For a great many hours after the clamping of the ureters the blood is still filtering through the kidneys. So it is not an instantaneous change?

DR. HUGHES: It should be provided you immediately stop the glomerular filtration. Actually I waited four hours for the kidney to swell and flow presumably to stop before injecteing the labeled albumin.

DR. GOODMAN: If you reimplant the ureter, it is really not a good control, you then have filtration going on.

DR. HUGHES: I believe it should be an excellent control. Then you should get the normal curve.

DR. DANOWSKI: It would be a good control.

DR. HUGHES: You are not losing anything from the body.

DR. EDER: Therefore it would be a control for uremia.

CHAIRMAN KRETCHMER: To conclude the discussion on the transport of macromolecules we are going to hear an introductory paper on the "Ultrafiltration and Excretion of Lipoproteins" which is under the authorship of Leon Hellman, Benjamin Kramer and Dr. Kurt Stern. This will be presented by Dr. Kramer.

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*Since the meeting Drs. Hughes and Eder have agreed that 8 grams per day is reasonable for the human.

D. Ultrafiltration and Excretion of Lipoproteins

DR. KRAMER: The brief communication to be presented contains some preliminary findings obtained in collaboration with the late Dr. Kurt Stern of the Brooklyn Polytechnic Institute whose premature death interrupted physico-chemical studies of the character of the urine and serum protein in the nephrotic syndrome. It has been known from the electrophoretic observations of Longsworth and MacInnes, Dr. Stern, and numerous other investigators that the urinary protein pattern in nephrotic urine differed markedly from that simultaneously observed in the plasma. The plasma contains low albumin and high globulin concentrations whereas the pattern of the nephrotic urinary proteins resembles that of normal serum. It was obvious therefore that the kidney in some manner functioned as a molecular filter. The more recent studies of the excretion of dextran preparations of various molecular weights by Brewer and also Wallenius seem to suggest that increased glomerular permeability rather than diffusion or tubular reabsorption of protein is responsible for the character of the proteinuria. The current studies were undertaken with Dr. Stern in order to determine *whether retention of the relatively poorly filtered protein fractions in the face of loss of* the more readily filterable proteins might not in part, at least, explain the observed elevation in the lipid fractions of nephrotic plasma which are usually associated with the proteins of the highest molecular weight. The globulins of smaller molecular weight such as the alpha-1 and gamma globulin usually are present in lower relative concen-t [illegible] even among the larger b [illegible] more sharply reduced t [illegible] l retention of the larger molecules would then provide a partial explanation for the elevation of some of the plasma constituents.

Figure 5 shows paper electrophoretic patterns of the urinary and plasma proteins of a nephrotic patient, stained so as to give information concerning protein and lipid content. It can be seen from the upper left diagram that the plasma alpha and beta globulins were elevated and from the lower left diagram, stained for lipid with Oil Red O, that most of the lipid material was in the alpha and beta regions. On the upper right portion of the slide are the urinary proteins obtained simultaneously. The urines were concentrated so that the amount of protein placed on a strip was the same for both plasma and urine. It will be seen that the alpha and beta globulin are relatively reduced as compared to the serum whereas the urinary albumin is increased. In the lower right diagram is the densometer reading of the identical strip stained for lipid with Oil Red O. It will be seen that the urinary proteins were almost devoid of lipid indicating that the kidney had in some manner largely filtered only those proteins which contained very little lipid. That this finding was not an artifact was subsequently shown in control experiments in which the nephrotic serum shown in the upper left was added to normal urine and the resulting electrophoretic pattern was virtually identical with that of the original serum both as regards protein and lipid distribution and concentration. This indicated that lipoproteins were not split after their excretion into the urine. Chemical analysis of nephrotic urine for cholesterol showed that despite the large quantities of protein present and the elevated plasma cholesterol level, that little or no cholesterol was present in the nephrotic urine. We next attempted to reproduce these *in vivo conditions by in vitro* ultrafiltration. These experiments were carried out with a Schleicher and Schuell Theissen-Ultrafiltration apparatus using very dense

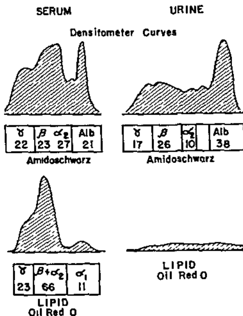
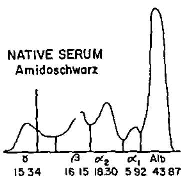
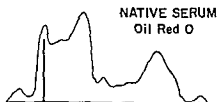
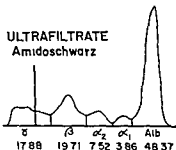


Fig. 5 Distribution of proteins and lipids in nephrotic serum and urine shown by paper electrophoresis and selective staining.







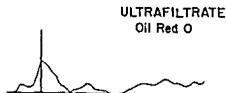


Fig 6 Ultrafiltration of serum proteins.

Fig 7: Ultrafiltration of serum lipids.

filters with nitrogen pressures of 15-25 pounds. These filters have pore sizes which are below 0.2 microns.

Figure 6 shows the protein pattern of the original serum as well as the ultrafiltrate derived from this serum. It will be noted that the changes in protein concentration are not remarkable except for the globulin reduction. The ultrafiltrate was concentrated as in the case of nephrotic urine in order to apply the same total amount of protein to the paper strip.

Figure 7 shows identical strips to those just shown but in this case stained for lipid with Oil Red O. The marked reduction in the quantity of lipid material which passes through the filter is quite evident. These slides are representative of a larger number of experiments which have been carried out with essentially similar results. The ultrafiltration technique is a difficult one because of the development of leaks in the membrane and a large number of fruitless experiments must usually be carried out for each successful run.

It was our intention together with Dr. Stern to further characterize the nature of the ultrafiltrate by ultracentrifugation as well as tracer and immunological studies. Unfortunately, we have not as yet had the opportunity to proceed with this intention. It is our hope that this brief presentation may suggest that this line of approach justifies further investigation.

DR. BOTT: I am not familiar with the characteristics of these membranes. Is it a more porous membrane than, for instance, cellophane? It probably is.

DR. KRAMER: Dr. Stern studied the permeability of the individual collodion membranes which he used and he had a great many membranes of varying porosity. He picked this one because it permitted the proteins to go through. So in the pattern the size was such that the smaller protein molecules went through but the larger ones were held back to a large extent.

CHAIRMAN KRETCHMER: Dr. Kramer, am I right in assuming that your final conclusion is that the ultimate protein filtrate attained with this membrane resembles the type of protein pattern that one would expect in the urine of an individual with the nephrotic syndrome?

DR. KRAMER: I am thinking along that line but not saying it.

CHAIRMAN KRETCHMER: Certainly the amount of albumin is lower than what one sees clinically.

DR. RAPOPORT: May I ask a general question? What has ever happened to the double refractile bodies that were found in the samples of urine which all medical students would tell you about? Dr. McCrory sort of laid the ghost. These bodies were said to be cholesterol and he could not find any fat in the urine.

DR. KRAMER: There is a little cholesterol.

DR. GOODMAN: I wonder if the findings are on the supernatant of centrifuged urine. There is a lot of lipid in the sediment.

DR. KRAMER: These urines were centrifuged.

DR. GOODMAN: The lipid you are speaking of would be in the cells at the bottom?

CHAIRMAN KRETCHMER: When urine is exhaustively extracted only meager amounts of lipid are obtained. This introduces the perplexing problem of how lipid resulting from lipoproteins is transported.

DR. McCRORY: We have been unable to demonstrate significant amounts of cholesterol, phospholipid or other lipid in the urine in nephrosis. The fact that the urine in nephrosis is essentially free of cholesterol was reported by Mirsky in the 1930's. This finding provides additional evidence of the differences between the serum and urine globulins in nephrosis. The fact that there is morphologic evidence of lipid in urine, and that lipoid bodies can be seen in urine and in renal tubules, is not contradictory. Quantitatively the urinary lipid is insignificant but if you look carefully you can see occasional lipoid bodies. Lipoproteins are not excreted in the urine in significant amounts.

DR. GOODMAN: No disagreement. The lipid is in the sediment, but the total would still be a small amount.

DR. SCHEINBERG: I did not understand your comment, Dr. Kretchmer. I thought I got the opposite conclusion from the early slide. I think Dr. Kramer's first slide showed a difference in the distribution of lipid in urine on electrophoresis, and in serum, whereas the ultrafiltrate when concentrated to the same level that urine had with respect to protein showed the same pattern when stained by Amido Schwarz, which to me meant that the ultrafiltrate was not representative of what the urine showed. Am I wrong?

CHAIRMAN KRETCHMER: I was talking about a different thing. I merely mentioned the lack of lipid in the urine of an individual with the nephrotic syndrome. The results obtained with the Schleicher and Schuell membrane are obtained from a static situation. This membrane has a fixed porosity which permits rather large molecules to permeate.

DR. SCHEINBERG: It does not change the pattern of what gets through at all?

CHAIRMAN KRETCHMER: It removes the more dynamic portion of the nephron, namely, the tubule.

DR. SCHIFF: In other words, the finding of a lower lipid content in the urine could just as easily be explained on the basis of differential tubular reabsorption of that moiety.

DR. HEYMANN: The lipid situation is very similar in ascitic fluid of nephrotic children. The concentration of total lipids and cholesterol is low.

CHAIRMAN KRETCHMER: One theory for the handling of lipoproteins by the kidney is that the lipid is hydrolyzed from the protein at the tubular membrane[30]. The lipid is then absorbed by the cells and the protein portion is excreted. I believe that double isotopic labelling will be necessary to test this theory.

DR. OLIVER: Isn't the electrophoretic pattern in the urine of this nephrotic patient as compared to the electrophoretic pattern of the serum quite different from that which Sellers et al.[31] find in the normal proteinuria of the rat in which the albumin is practically absent from the urine; that is an observation that had been confirmed by Cochran et al.[32] and was indirectly confirmed by Rigas and Heller[33] and others who have shown that in the normal proteinuria of man there is a reduction in the albumin.

DR. KRAMER: I think Dr. Goodman can talk for Dr. Sellers.

DR. GOODMAN: You are right.

In humans the normal proteinuria is about 30 to 60 mg. per day and is almost all globulin. This is used as indirect evidence for the reabsorption of albumin, assuming that both albumin and globulin were filtered through the glomerulus. But here we are dealing with massive proteinuria. Not only has albumin appeared, but much more globulin.

DR. OLIVER: In other words, the situation in the nephrotic has changed from the normal and the difference might be explained in part by saturation of the tubular mechanisms that no longer absorb albumin.

DR. GOODMAN: As well as the possibility that an increase in glomerular permeability has occurred.

DR. FOX: While you are using the word "proteinuria", I wonder if everyone is aware of the large amount of mucoproteins present in the urine. There is considerable protein-like material which is not coagulated by heat and not precipitated by most of the usual protein precipitants. Maclagan and Anderson[34] have made extensive studies of the urinary mucoproteins by isolation and by the diphenylamine reaction.

There is a sizeable concentration in the plasma and urine and the molecular weights have been measured. In discussing "proteinuria" it might be wise to modify the terminology and remember we are talking only of proteins precipitated by the ordinary protein precipitants. There is a sizeable quantity, over 100 mg., of mucoproteins which are excreted daily in normal human urine.

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[30] Reubi, F. and Schmid, A., Jour. D'Urol. Suppl. Vol. 61, 304, 1955.
[31] Sellers, A. L. et al., J. Exp. Med., 95:465, 1952.
[32] Lewis, L. A. et al., Amer. J. Physiol., 180:331, 1955.
[33] Rigas, D. A. and Heller, C. G., J. Clin. Invest., 30:853, 1951.
[34] Anderson, A. J. and Maclagan, N. F., The Isolation and Estimation of Urinary Mucoproteins. Biochem. J., 59:638, 1955.

CHAIRMAN KRETCHMER: Considerable amount of work has been accomplished at Brookhaven in regard to TCA soluble protein.

DR. HUGHES: Dr. Popenoe at Brookhaven has been interested in the alpha-1 glucoprotein, which is not precipitated by TCA. However, I would like to emphasize that I would expect small amounts of many serum globulins in urine as well as albumin. Their amounts would depend on the glomerular permeability and hence would vary from species to species. Of course, they should be elevated in renal disease. However, due to their wide variation in properties, I think the use of the word globulin is not sufficiently informative and permits a variety of interpretations.

DR. McCRORY: There is a considerable amount of protein in these urines that is perchloric acid precipitable but not precipitated by trichloracetic acid. The alpha-1 glycoprotein would behave in this way.

DR. OLIVER: The presence in the urine of glycoprotein would still constitute a "proteinuria". I might add that they always show up histochemically in PAS preparations not only in the plasma in the blood vessels from which they are derived, but also in the filtrate in the tubule lumens and are greatly concentrated intracellularly in any protein absorption droplets that may be present.

DR. McCRORY: Your point is well taken. We have attempted measurements of the urinary glycoproteins. The glycoproteins do contribute to the estimation of electrophoretic fractions when dye staining (Amido-Schwarz) is used to estimate fractions. We have found the glycoprotein content of nephrotic urine to be around 0.-0.2 gm.% (measured as polysaccharide) in urines containing total protein (trichloracetic precipitate) of 2-3 gm.%. The glycoproteins would not bind protein dyes to the same degree per gram of protein as the other urine proteins because of their low protein content. Consequently the error would probably be small in relation to the estimation of the separate fractions. I am more interested in a different point. I find your pattern of the "ultrafiltrate" of plasma quite different than the typical pattern of nephrotic urine. The albumin in nephrotic urine comprises 60-80% of the protein even though the amount in serum is very low. The per cent albumin in your ultrafiltrate is not too different from the serum. I would like to get Dr. Blainey to comment on the ultrafiltration studies I saw this summer that are being done in his department in Birmingham.

DR. BLAINEY: Dr. David Rowe in our department has been doing experiments essentially similar to those that we have just heard about using collodion membranes that he makes himself. These membranes can be made with different pore sizes depending upon the concentration of alcohol-water mixtures in which the membranes are conditioned. With membranes of suitable pore sizes it is possible to modify the serum by ultrafiltration to be indistinguishable from the electrophoretic pattern seen in the nephrotic syndrome, and also to obtain ultrafiltrates that are remarkably like nephrotic urine.

DR. GOODMAN. Does the lipid come through?

DR. BLAINEY. No, not at all.

DR. BARNETT: You did not prepare tubules?

DR. BLAINEY: No.

DR. FOX: Aren't you going to have a great discrepancy between the total protein and the electrophoretic pattern because the glycoproteins will be represented in electrophoretic studies, whereas they won't be represented in the analytic studies?

DR. McCRORY: The per cent composition of the electrophoretic patterns are estimated by dye staining and would include the glycoproteins and mucopolysaccharides. The total protein estimation, when done by conventional methods (trichloracetic acid precipitation) would not include this component. Consequently, some error would be introduced but I believe it is not a large discrepancy.

DR. FOX: Maclagen[35] found in some diseases, especially inflammations, that there is an increased urinary excretion of mucoproteins.

DR. McCRORY: We found that the published methods that presumably allowed one to get glycoproteins, in our hands did not give us anything except a general measure of the glycoproteins.

DR. FOX: Did you use the diphenylamine reaction?

DR. McCRORY: No. I am not familiar with that but again we got a population of polysaccharides, not a single component.

DR. NORMAN ZEIG: An interesting approach as to what can be filtered can perhaps be given by Dr. Bott, if she can analyze the various protein fractions that one can get in the filtrate from a single glomerulus. That this is difficult is obvious.

DR. BOTT: We have not reached the stage of electrophoresis on glomerular filtrate. It is rather interesting in this connection, though, that we treated cellophane membranes with zinc chloride[27] in order to increase the so-called pore size and we were able to get the same percentage of protein through, for instance in the case of egg albumin, as we got through the glomeruli in amphibia, but we did not find the same differences in the filtration percentage of various proteins which had similar molecular weights as we found in the actual glomerular filtration. For instance, the egg albumin, which has about the molecular weight and size of lactoglobulin, did not come through the glomerulus in quite as great concentration as the lactoglobulin did. But if we prepared a membrane that would let egg albumin through as much as did the natural membranes, it also let the lactoglobulin through in that same concentration. There seemed to be a difference -- whether it was a matter of charge or shape -- that the animal membrane could distinguish between the two and the artificial one could not.

DR. HUGHES: The difference is a factor of two?

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[35] Lockey, E., Anderson, A. J. and Maclagan, N. F., *Urine and Serum Mucoproteins in Cancer and Other Diseases*. Brit. J. Cancer, 10:209, 1950.

DR. BOTT: Not that much. I think it was about 20 per cent.

DR. LANGE: Where your hole is just big enough to let the egg albumin fall through, only the egg albumin will fall through. If the hole is a little bigger both egg albumin and lactoglobulin will fall through.

DR. BOTT: As we put it, we figured about half of the membrane had a pore size diameter large enough to let egg albumin through.

DR. LANGE: Large enough or larger?

DR. BOTT: I don't know that we could say that definitely. We did try proteins of different sizes but I don't have all the information in my mind at present. Then we tried with proteins like lactoglobulin and I believe the figure there was about 75 per cent. As far as we knew at that time there was very little difference in molecular size. There was a little difference in shape. That might have accounted for it but the artificial membranes did not distinguish between them.

DR. METCOFF: Is there any evidence that proteins are not secreted into the tubular lumen?

CHAIRMAN KRETCHMER: Only the evidence of the classical experiments of Bieter where he showed that there was no protein in the urine of aglomerular fish.

DR. EDER: I should like to know if there are any plasma proteins as large or larger than alpha-1 lipoprotein with a molecular weight of 200,000 or beta-1 lipoprotein with a molecular weight well over 1,000,000 which get through the glomerular membranes.

DR. HUGHES: Fibrinogen does not get through.

DR. EDER: What is the molecular weight?

DR. HUGHES: 500,000 but it is very asymmetric.

DR. EDER: The molecular weights of proteins which pass through the glomerular membrane are well below those of the lipoproteins.

DR. HUGHES: The shape factor must be very important in considering permeability since a molecule is going to be pretty randomly oriented going through a hole. Therefore its effective diameter should be related more to the length than to the width of the molecule. This may explain why the spherical hemoglobin molecule passes the glomerular membrane more readily than serum albumin which has the same mass but is asymmetric.

DR. JOSEPHSON: The molecular size was determined by G. Wallenius[36], who injected dextran in man and dogs and measured the size of the largest body which could

[36] Wallenius, G., Renal clearance of dextran as a measure of glomerular permeability, Acta Societatis Medicorum Upsaliensis, Suppl. 4, Uppsala 1954.

pass the glomerular membranes. He could actually show (page 64) that the size of the molecule passing was much higher in the nephrotics than in normal man.

DR. HUGHES: I am sure that was true.

CHAIRMAN KRETCHMER: I would like to spend a few moments summarizing the discussion. I was impressed this morning and afternoon that most of us were convinced that protein can be taken up by the tubule cell. This fact was well demonstrated by Dr. Oliver's material. The data presented on the clearance of proteins leaves us a little perplexed. If a negative intercept does really exist, then certainly protein has been demonstrated to be absorbed by the tubule cell.

Once the protein is in the cell something happens to it and we were shown data which indicated that the kidney was important in the degradation of protein and, therefore, participates in the overall problem of protein metabolism.

We remain with many unanswered questions, however, that is the function of a symposium of this type. We in reality have no conception of the mechanism of protein absorption. As a matter of fact, we have very little information concerning the absorption of the amino acids. There are only fragmentary ideas of the relative selectivity of the tubule cell membrane for the proteins such as hemoglobin, albumin, or the various globulins.

We would also like to have information concerning the relation of protein absorption to the total economy of the organism. Suffice it to say, as clinicians we are still left with doubt as to the mechanism for proteinuria. However, I believe that the answer will come in the near future.

II. TRANSPORT OF MICROMOLECULES IN MAMMALIAN AND NECTURUS NEPHRONS

CHAIRMAN COOKE: Dr. Barnett asked me to supervise the discussion this afternoon in regard to the "Transport of Micromolecules in Mammalian and Necturus Nephrons," and I am anxious to find out exactly where the macromolecule stops and the micromolecules begin. It sounded like Dr. Bott was involved with a little bit of each and perhaps she could open the discussion.

A. Evidences from the Concentrations of Electrolytes in Tubule Fluid, Serum and Urine, Especially in Amphibia

DR. PHYLLIS A. BOTT: I shall not confine my remarks entirely to the amphibian kidney. I understand that this is a very informal sort of meeting and I shall be glad to stop at any time but I intend to go over some of the earlier work first very briefly. I am sure some of you, especially those who are clinicians, have not been following this work closely enough to realize some of the developments.*

Just to acquaint you with the story and the problems that it has led up to: the general techniques which we have used in our work are, of course, those developed by Wearn and Richards[1], and Richards and Walker[2], and in those few that I shall refer to that were done on mammals, the technique of Walker and Oliver[3] for collection and identification of point of puncture.

It was back in about 1937 when I was still with Dr. Richards, realizing the importance of the fixed bases in the electrolyte picture, that I began to try to develop methods for the determination of sodium and potassium. Pictures explaining the sort of capillary apparatus that was used in working out some of these methods were published in 1943[4].

In the capillary apparatus used for the determination of sodium a precipitate of sodium zinc uranium acetate was thrown down from a fine capillary tube to a filter, filtered off, washed and dissolved. The solution was made up to a volume of 6 ml.,

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* For the past five years these studies have been supported by grants from the Life Insurance Medical Research Fund.

[1] Wearn, J. T. and Richards, A. N., Am. J. Physiol. 71:209, 1924.
[2] Richards, A. N. and Walker, A. M., Am. J. Physiol. 118:111, 1937.
[3] Walker, A. M. and Oliver, J., Am. J. Physiol. 134:562, 1941.
[4] Bott, P. A., J. Biol. Chem. 147:653, 1943.

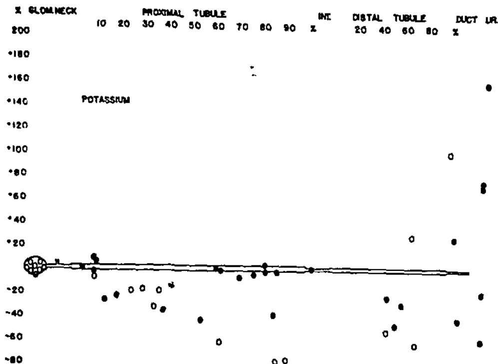


Fig. 8: Reabsorption of potassium in amphibia.
O = results of early experiments (chemical determinations)
● = recent flame photometer results.

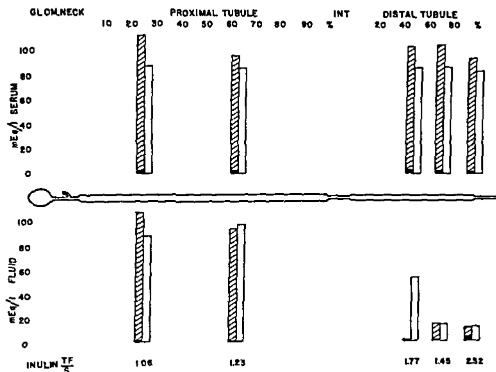


Fig. 9: Comparison of electrolyte concentrations in serum and various levels of tubular fluid. Sodium cross-hatched; chloride open and potassium, black columns. Values for serum are above the nephron; tubule fluid values shown below it.

treated with dithizone and the sodium finally determined as zinc in this large volume. With this method the concentration of sodium in glomerular filtrate in Necturi was determined and was found to average 1.7 per cent below that of serum with a variation of about 7 per cent. This sodium method was finished or nearly finished at the time that the series of mammalian experiments in which Dr. Walker made the collections[5] was being completed. Fortunately I was able to run sodium determinations on two of these rat kidney tubule fluids on which chloride was also determined.

The ultramicro method for potassium[6] involved the precipitation of the element as the familiar sodium potassium cobaltinitrite. This was a much finer precipitate and harder to handle. I could not filter it off and finally resorted to throwing the fluid containing the precipitate down from a fine capillary tube into a slightly larger "U" tube which was made of semi-transparent plastic. One short and one long glass arm were attached to the "U". Fluids were mixed by attaching a "pusher" to the long arm. Wash fluids were admitted by way of the short arm and removed by exerting pressure on the long arm. The precipitate was dissolved in water, and the potassium determined colorimetrically as nitrite with alpha naphthylamine and benzidine in a volume of about 1 ml.. With this method the concentration of potassium in glomerular fluid, again in Necturus, was found to average about 1.6 per cent below that of serum, with a variation of about 3 or 4 per cent. The method seemed to be working fairly well.

At about this time a great interest in potassium arose because the results of indirect experiments performed by two different groups of workers had suggested that potassium was absorbed in the proximal tubules[7, 8], one of them[8] suggesting that the fluid might even be rendered practically free of potassium in going through the proximal tubule. I had been running these experiments on tubule fluid of amphibia and had prepared to give a preliminary report of them before the Physiological Society of Philadelphia[9]. I picked up the slides on my way to Princeton where the 1953 Macy Foundation Conference was being held, knowing that Dr. Berliner and several people associated with Dr. Mudge, who were the investigators whose work I just mentioned, would be there and would be interested in them. Perhaps against my better judgment I did show the slides and because there was much enthusiasm about them I allowed them to be printed[10].

My first experiments indicated that potassium was practically completely filtered and that the concentration fell off in the proximal tubule. These results are represented by open ovals in Figure 8. The distal tubule figures sometimes indicated concentrations much lower than that of serum but a few were high. Inulin ratios, not shown here, indicated that water was being reabsorbed, although there was considerable variation from

[5] Walker, A. M., Bott, P. A., Oliver, J. and MacDowell, M. C., Am. J. Physiol. 134.580, 1941.
[6] Bott, P. A., J. Biol. Chem. 215:287, 1955.
[7] Berliner, R. W., Kennedy, T. J., Jr. and Orloff, J., Am. J. Med. 11:274, 1951.
[8] Mudge, G. H., Ams, A., Foulks, J. and Gilman, A., Am. J. Physiol. 161:151, 1950.
[9] Bott, P. A., Proc. Physiol. Soc. Phila. (October 20, 1953). See Am. J. Med. Sci. 227:102, 1954.
[10] Bott, P. A., Trans. 5th Conf. on Renal Function, Josiah Macy Jr., Found., New York, 1954.

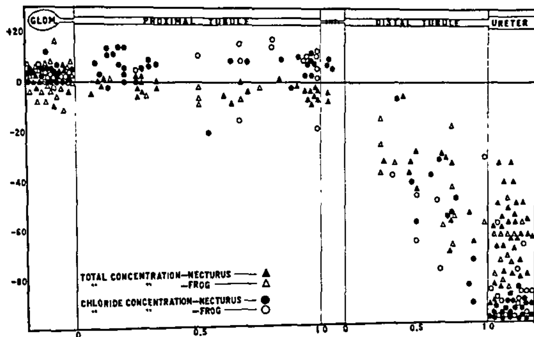


Fig. 10: Ratios of total solute concentrations and of chloride concentrations in serum and urine in amphibia[11].

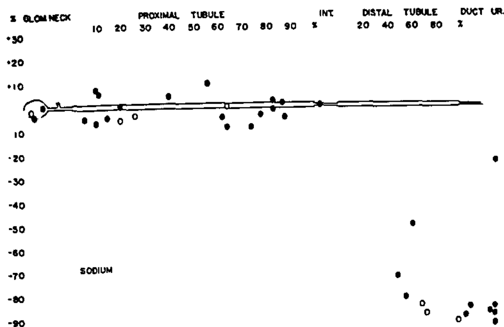


Fig. 11: Reabsorption of sodium in amphibia.
O = older chemical determinations
● = flame photometer results

experiment to experiment. Each dot means a separate experiment. I warned that these should be considered as preliminary experiments and that I thought there might be a spread and more of the values for the proximal tubule would go off in the direction indicated by the distal tubule figures.

In Figure 9 are shown some experiments in which inulin, potassium, sodium and chloride were done. These were shown, also, at the 1953 conference. Sodium is shown as the cross-hatched, chloride as the open and potassium as black columns. Those above the nephron are for serum and those for the tubule fluid are below, the point of collection being shown by the position. Each tubule fluid pattern must be compared with that for serum from the same animal shown directly above it. The inulin concentration ratios are given at the bottom. The chloride seemed to rise to meet the sodium value, which was staying about the same throughout the proximal tubule and then chloride and sodium fell off in the distal tubule. It was brought out at that meeting, that this would seem to indicate (if the sodium stayed constant and the chloride came out higher than the serum value) a drop of pH in the proximal tubule, and I had to admit that it looked as though it was true. I was not surprised to find, as for instance in the collection made at about 60 per cent proximal, that the chloride ratio was higher than 1, because that had been found in Dr. Richards' laboratory before [11]. Figure 10 shows the results obtained there. My ratios on the whole have not been as high for chloride as those that Walker, Hudson, Findley and Richards had obtained. They performed the experiments from which these data were collected. The results are totally uncorrected. The osmotic pressure was found by them to remain essentially the same throughout the proximal tubule. Although my chloride ratios were not as high as most of theirs many have been found to be higher than 1 in the proximal tubule. Since the sodium was staying the same as in serum, and the osmotic pressure (according to results in Figure 10) was staying the same, unless there was some change in pCO_2 there should be a drop in pH. But in another series of experiments performed in Dr. Richards' laboratory, Montgomery and Pierce [12] had not found a drop in pH in the proximal tubues of amphibian kidneys. The two situations did not fit. This worried me so that we finally got back to it in my laboratory last year and I shall describe this work later.

Going on to more of the sodium and potassium experiments: In 1953 I ordered to be built* a flame photometer of the type described by Ramsay and co-workers [13]. I hoped that this would speed up our work and make it more accurate. It has taken several years to bring this photometer to the point where it is satisfactory for this work. In the first year of this interim period Dr. Heinrich Wirz was in our laboratory and together we ran some experiments [14] in which we determined the concentration of potassium and glucose in the tubule fluid of rats. This again was done by ultra micro-chemical methods, except for the urine potassium which was done by "macro" flame

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* Built by the American Electronic Laboratories, 121 N. 7th Street, Philadelphia.

[11] Walker, A. M., Hudson, C. L., Findley, T., Jr. and Richards, A. M., Am. J. Physiol. 118:121, 1937.
[12] Montgomery, H. and Pierce, J. A., Am. J. Physiol. 118:144, 1937.
[13] Ramsay, J. A., Brown, R. H. J. and Falloon, S. W. H. W., Jr. Exp. Biol. 30:1, 1953.
[14] Wirz, H. and Bott, P. A., Proc. Soc. Exp. Biol. & Med. 87:405, 1954.

TABLE 1.

POTASSIUM IN RAT TUBULE FLUID

Results of Analyses of Proximal Tubule Fluid

No.	Man., ml.	Collection: Site*	Amt., µl	Rate, µl/hr.	Potassium: Fl. mEq/l	P.	Fl/P.	Glucose: U/P.	Fl. mg/100 ml.	P.	Fl/P.	Urine Rate, ml/hr.
1	2	3.9/11.8	.58	.39	5.56	6.47	.86	.70	182	225	.71	.25
2	3	4.5/13.4	.35	.66	4.40	5.51	.80	4.14	110	364	.30	.30
3	2	4.1/10.1	.22	.12	4.08	7.06	.58	1.77	---	---	---	.06
4**	1	5.2/11.7	.41	.41	2.12	4.92	.43	19.00	136	275	.49	.40
5**	2	5.3/11.6	.50	.50	2.05	2.76	.74	20.30	266	348	.76	.60
6	3	4.3/ 9.4	.25	.15	3.64	4.34	.84	1.13	---	---	---	.25
7	3	4.8/10.1	.15	.10	1.48	5.74	.26	1.66	---	---	---	.17
8	2	6.0/11.9	.11	.08	3.44	6.05	.57	.57	---	---	---	.10
9	2	5.7/ 9.9	.57	.36	5.48	4.82	1.14	4.18	198	279	.71	.19

Man. = 5% manitol injected intrav; Fl. = tubule fluid; P. = blood plasma (a correction of 7% has been added to account for plasma proteins), U. = ureteral urine.

* Numerator of the fractions in this column is the measured distance, in mm., from the beginning of the *convolution to the* point of the fluid collection; denominator is measured total length of the convolution.

**Unilaterally nephrectomized animals.

photometer. The results, shown in Table 1 indicate that potassium was reabsorbed in the proximal tubule and with one exception, the ratios were all lower than one. However, I would like to point out that we did have one in which we had a ratio of 1.14. If you realize that the reabsorption of water, as shown before in the experiments on mammals[5], indicated that the concentration of inulin -- had we had it here -- would be much higher, probably there is reabsorption of potassium indicated there but the ratio is higher than one. In other words, the concentration has not dropped below that of serum in this experiment. We determined glucose at the same time because I had become interested in whether or not potassium and glucose were involved in the same absorption mechanism. In some cases the ratios for these two substances agree and in some cases they do not agree. As far as the secretion of potassium is concerned, these experiments did not prove that we were demonstrating secretion. However, the strikingly higher increase in potassium concentration ratios between proximal tubule and urine in the unilaterally nephrectomized animals is suggestive of secretion there.

If I may, I should like to say a few words about our flame photometer, of the Ramsay type. I am still trying to determine all of the parameters of the instrument but it will detect as low as two micromicromols of either sodium or potassium -- even less probably, of sodium. It determines sodium and potassium simultaneously. I don't want to take too much time to discuss it but if anyone is particularly interested in the mechanism of it I shall be glad to discuss it later.

TABLE 2

Comparison of Macro and Micro Flame Photometer Analyses

Material		Potassium mEq/l.		Sodium mEq/l.	
		Ramsay Ultramicro	Fox "Macro"	Ramsay Ultramicro	Fox "Macro"
"Unknown"					
4.0 K + 102	Na	4.1		101	
Necturus serum	No. 1	3.5	3.3	94	90
" "	No. 2	4.0	4.0	92	93
" "	No. 3	4.2	4.0	84	89

Table 2 shows some figures for macrodeterminations compared with the ultramicro. Miss Sarah Mills, who is assisting me in this work, made the "macro" determinations. For the ultramicro analyses a 20-fold dilution was made first, both on the unknown and the serum and then two tenths of a microliter of serum was used for the determination. The amounts concerned here in the final samples were about 2 millimicrograms of potassium and about 50 of sodium. The only reason sodium is so high is because that is the way it is in serum and we had to be able to determine them simultaneously. These are determined simultaneously in one flash as the wire goes through the flame. For the present, three readings are usually made on each sample. This then shows the type of errors I was getting last year[15] which included microdilution, errors of pipetting samples on the wire, etc., and there was fairly good agreement between micro and macro and good agreement on the unknown.

[15] Bott, P. A., Fed. Proc. 15:223, 1956.

Figure 11 shows sodium figures essentially as presented last spring[15]. Open ovals represent the older chemical determinations and the black ovals are flame photometer results. The experiments seem to indicate that the sodium concentration does not change much during the passage of the fluid down the proximal tubule. There may be just a slight hint that it is beginning to drop off as the chlorides did right at the end of the proximal tubule but I am not sure of it yet and we are trying to fill in the intermediate space now. In most experiments on amphibia the value for sodium drops very low in the distal as it does for chloride also. We can say that in the proximal tubule the sodium concentration seems to stay the same, whereas in the distal tubule it drops off to very low values. I might say in passing that in those two experiments in which I was able to analyze for sodium on the rat kidneys[5] also indicated that at least a third of the way down the proximal tubule the concentration of sodium was again very close to that of serum so it may be that the same sort of thing is happening there.

CHAIRMAN COOKE: Was this for potassium or sodium?

DR. BOTT: This was sodium in the rat kidney. We have only two figures so far.

Returning to Figure 8 showing the potassium: As indicated earlier the older, chemically determined figures are shown as open ovals. The flame photometer figures not previously mentioned today are represented by black ovals. As in the earlier experiments some of the newer distal tubule fluids showed a lower concentration of potassium than serum while some showed a higher concentration. Urine concentrations are also either higher or lower than that of serum. The first point of disagreement between old and new results is in the proximal tubule. At first I did not care how these things came out but having presented the first experiments, even though I warned they might not all come out that way, I was disappointed too when I found that more and more were coming out with no drop in potassium concentration, even close to the end of the proximal tubule. These figures puzzle me. I am not prepared to say whether either type is wrong as yet. I think they should serve as a warning, however, not to assume too much that potassium is reabsorbed completely in the proximal tubule. Certainly there seems to be indication here that in many of them the concentration may stay the same and you remember in one of the rat experiments we also showed that one tubule fluid had a higher potassium than the serum concentration.

Whether or not there is some reabsorption of sodium or potassium in the proximal tubule of Necturi when the concentration ratios of these elements are close to 1 depends, of course, upon the inulin ratios determined simultaneously. Before showing these relative ratios I would like to show a chart (Figure 12) made by plotting all of the inulin ratios for collections that I have made to date on Necturus* because there was some doubt, not only in the minds of others but actually in my mind too about whether or not there really was reabsorption of water in the proximal tubules of these animals. Very often the ratio is low. I think those shown below the nephron are within the total error of the experiments, and in fact the dot at 10% proximal shows a slight

* These include some from earlier work[16] and more recent studies in which zinc hydroxide was used for precipitation of serum protein.

[16] Bott, P. A., Am. J. Physiol. 168:107, 1952.

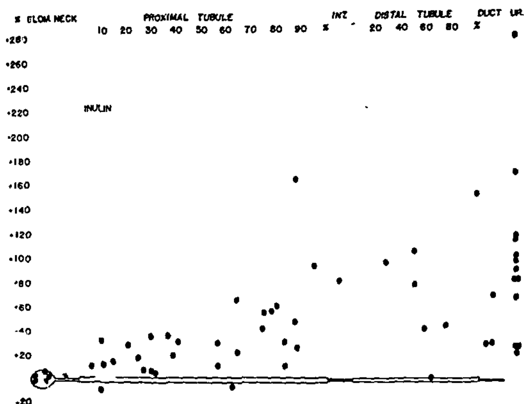


Fig 12. Inulin (filtered/serum) ratios in necturus nephrons, illustrating water reabsorption.

error toward the negative side. Many times the inulin becomes concentrated and I think especially when you can see a progression of points of this kind there is no doubt that some water is reabsorbed in the proximal tubules of many of these animals. I think when you find in the urine of the animals a fairly high ratio such as some of the higher ones on the chart you are almost bound to find some concentration of inulin in the proximal tubule. The highest point on the chart signifies a ratio of 3.8. This picture looks very much like that of Walker and Hudson[17] for glucose after phlorhizin which was supposed to measure the same thing. I think it is fairly definite that the inulin ratio means something and that the ratio of sodium or potassium, as compared with inulin therefore means something. If we don't believe there is reabsorption of water, then we cannot say that there is reabsorption of sodium. For those who are interested in comparing these ratios, of course a dot at +41 per cent represents a ratio of 1.41, +80 represents 1.80 and one at about +170 a ratio of 2.7. This last is very unusual for proximal tubule of Necturus after injection of inulin solution but we have had a number of fairly high ratios at the end of the proximal tubule.

The relative ratios of the various substances are shown in Table 3 for some experiments in which potassium did not show a drop in the proximal tubule. All were determined simultaneously at various proximal tubule puncture sites. I called attention to the dot on the inulin chart representing the first experiment listed for a 10 per cent proximal puncture site. I think there is a slight error in the inulin determination there.

TABLE 3

NECTURUS KIDNEY

Proximal tubule $\frac{\text{fluid}}{\text{serum}}$ concentration ratios

Puncture Site %	Inulin $\frac{TF}{S}$	$\frac{\text{K ratio}}{\text{In ratio}}$	$\frac{\text{Na ratio}}{\text{In ratio}}$	$\frac{\text{Cl ratio}}{\text{In ratio}}$
5	1.11	0.91	0.86	0.94
10	0.91	1.06	1.18	1.00
10	1.13	0.96	0.82	0.88
14	1.15	0.79	0.90	0.86
64	1.67	0.59	0.54	0.59
74	1.42	0.65	0.64	0.74
78	1.56	0.61	0.62	0.67
83	1.12	0.92	0.87	0.83
88	1.27	0.76	0.75	0.76

Because of this it then looks as though the inulin ratio is less than one, and it makes the other ratios high. I believe that the error is in the inulin determination but whether it is or not I have kept that figure in there. You notice that there are on the whole increasing ratios of inulin as the sites progress along the tubules, although occasionally there is one in which there is not much concentration. It seems to me (if you do throw

[17] Walker, A. M. and Hudson, C. L., Am. J. Physiol. 118:130, 1937.

out this one experiment) that there is no indication of secretion of sodium, potassium or chloride in the proximal tubule and what reabsorption there is of those substances seems to be along with water. Which comes first and which controls which cannot be told from these experiments.

In some of these I was able to collect urine from the ureter at the same time that I was doing fluid collections. Ratios for these are shown in Table 4. At the right of the table we have still no suggestion for secretion of either sodium or chloride. We do have a suggestion in two of them that there may be some secretion of potassium. There are so many pitfalls in this type of experiment that I am not prepared to say as yet that secretion had definitely been established but there is a suggestion of it and we must do a lot more work on it. On the left side of the table I have compared ureteral urine over serum ratios with the TF/S ratios. In other words, these figures show the increase in concentration ratios from a site in the proximal tubule to the ureter. Again in two experiments the increment in the potassium ratio is greater than that for inulin and it would seem that between this site and urine there was secretion (or some type of ion exchange that we call secretion) of potassium. This, of course, assumes a fair homogeneity of nephrons and requires many more experiments for actual proof.

Another thing that appears to be so from a comparison of these ratios is that the disappearance of sodium and chloride from the distal tubule fluid, (that is, between the proximal site and ureter) seems to happen at a much greater rate than the reabsorption of water and they run along in a sort of parallel manner. I perhaps should not say "disappearance of sodium and chloride". Perhaps I should say that the concentration drops off faster. We still have not proved that there is not secretion of water. They drop off faster than water is reabsorbed or the concentration would remain the same over this region and they drop off very much at the same rate in these examples.

TABLE 4

NECTURUS KIDNEY TUBULE FLUID - URINE RATIOS

Proximal Site %	(UU/S ratio) / (TF/S ratio) In	K	Na	Cl	K ratio / In ratio TF	UU	Na ratio / In ratio TF	UU	Cl ratio / In ratio TF	UU
5	1.11	0.81	0.07	0.08	0.91	0.66	0.86	0.06	0.94	0.07
10	1.99	1.77	0.71	0.09	1.06	0.95	1.18	0.42	1.00	0.04
74	1.41	3.92	0.15	0.09	0.65	1.80	0.64	0.07	0.74	0.03
78	1.33	0.42	0.13		0.61	0.19	0.62	0.06		
83	1.15	1.71	0.11		0.92	1.36	0.87	0.09		

To go back to the problem of why we have a high chloride and no change in pH: The experiments of Montgomery and Pierce[12] had indicated no change in pH in the proximal tubules. The experiments done at a different time by another group of investigators in the same laboratory[11] had shown the little hump in the chloride and the isosmotic tubule fluid. My experiments had shown that sodium remained about the same

concentration throughout the proximal tubule. For the reasons explained earlier these things did not fit. I was very anxious to try all of these things together but at the time the photometer that I wanted to use was not ready. At my suggestion Dr. Gerhard Giebisch, who was working with me a year ago, tackled the problem of collecting enough material in the electrode used for pH determination by the Montgomery and Pierce method with the hydrogen electrode so that he could determine chloride and inulin on those samples. He used the conditions used by Montgomery and Pierce, that is, an oil layer over the kidney and CO_2 bubbled through it at the proper pressure. I might say here that in my experiments generally, I have an oil layer on top of the kidney. *That was not true in all the experiments from Richards' laboratory.*

Table 5 shows the results of Dr. Giebisch's experiments [18]. The chloride ratios have all been put on a water basis, (a correction was made for the average protein concentration in serum). Corrected serum concentration of chloride comes out fairly close to that of the proximal tubule fluid concentration, but on the whole it still runs just a little bit higher than that of tubule fluid. The pH to our surprise came out exactly as Montgomery and Pierce had said. The pH did not drop significantly throughout the proximal tubule under the conditions of these experiments in this animal, while urine pH values were lower. These results, however, fit together much better than the two separately done sets of experiments that I quoted before.

Now I know that you are all interested in mammalian experiments. I might say in the mammalian experiments [5] the chloride hump was much more exaggerated than it was in the amphibian experiments. I believe it is real. From all I can determine going back through my notes on it, I cannot find a thing wrong with those determinations. Some of the results were determined on protein-free, that is, zinc hydroxide treated, filtrates. Dr. Giebisch felt that he was getting lower results on zinc hydroxide treated filtrates (indicated by asterisks in his table) but in the mammalian experiments the ratio still went up as high as 1.4, even after correcting for protein content, etc. If the sodium is staying the same, as I think it is, throughout a large part of the proximal tubule we may have a different situation there and I think it is quite possible that we may have a change in pH that has not yet been determined.

DR. FOX: What is the remaining anion pattern, those chlorides being about 25 meq. lower than you see them in the human? What is the sodium level?

DR. BOTT: You mean in serum?

DR. FOX: Yes.

DR. BOTT: One set I can remember averaged a little over 100 and another set ran a little below 100 for sodium. I think about 95 to 100 meq/l.

DR. SHIPP: We have noted quite a variation in the serum sodium which may reflect seasonal fluctuations. Analyses in May and June of 1955 on the serum of 9 animals showed a sodium concentration of 97.5 ± 4.6 meq/l. The mean serum sodium was 84.8 in July 1955 and 83.5 in September of 1956. At any given time there is a considerable variation from animal to animal.

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[18] Giebisch, G., Am. J. Physiol. 185:171, 1956.

TABLE 5

INULIN, CHLORIDE AND pH VALUES AT VARIOUS LEVELS IN NECTURUS TUBULES

Exp. No.	Site	Collection		Serum Average		Fluid		Fluid/Serum		pH				
		Amt. µL.	Rate µL/hr.	Inulin mg.%	Cl. mEq/L.	Inulin mg.%	Cl. mEq/L.	Inulin	Cl.	Whole Blood	Fluid	Bladder or Uret. Urine	Δ Blood-Fluid	Δ Blood-Urine
9	Glom.	0.16	0.25	259	70.9	270	72.5	1.04	1.02	7.45	7.51	---	+0.06	----
	Prox.													
10	10%	0.20	0.83	355	76.8	381	78.6	1.07	1.02	7.50	7.49	6.05B	-0.01	-1.45
11	12%	0.59	0.89	108	73.2	150	74.0*	1.39	1.01	7.56	7.52	7.12B	-0.04	-0.44
12	18%	0.67	0.67	358	67.0	442	70.0*	1.23	1.04	7.51	7.59	6.94B	+0.08	-0.57
13	20%	0.15	0.30	297	80 9	434	84.0	1.46	1.04	7.44	7.46	6.12B	+0.02	-1.32
14	24%	0.19	0.49	248	83.2	311	90.2	1.25	1.08	7.46	7.45	6.89B	-0.01	-0.57
15	28%	0.57	0.98	431	76.8	490	70.0*	1.14	0.91	7.50	7.50	6.05B	0.00	-1.45
16	29%	0.42	0.63	245	88.1	300	92.0*	1.22	1.04	7.46	7.47	7.01B	+0.01	-0.45
17	33%	0.30	0.35	279	74.9	380	81.2	1.36	1.08	7.46	7.40	6.83U	-0.06	-0.63
18	43%	0.59	0.71	273	71.2	308	73.0*	1.13	1.02	7.51	7.56	7.17B	+0.05	-0.34
19	50%	0.06	0.08	---	---	---	---	---	---	7.50	7.46	---	-0.04	---
20	55%	0.42	1.15	504	75.7	565	74.5*	1.12	0.99	7.60	7.54	6.79B	-0.06	-0.81
21	58%	0.05	0.07	---	---	---	---	---	---	7.49	7.51	6.68B	+0.02	-0.81
22	87%	0.23	0.35	373	76.9	590	83.5	1.58	1.08	7.32	7.39	6.48B	+0.07	-0.84
	Ureter													
23	"	---	---	394	73.9	1480	6.9	3.76	0.09	---	---	---	---	---
24	"	---	---	750	75.1	1980	15.0	2.64	0.20	7.52	6.28	---	-1.24	---

DR. FOX: The total osmotic pressure of one serum is lower.

CHAIRMAN COOKE: Is there a difference between the early experiments in which the potassium fell to low levels and subsequent groups of experiments, except instrumentation?

DR. BOTT: As far as I know there is no difference.

CHAIRMAN COOKE: The season was the same? The animal would have been in comparable condition?

DR. BOTT: That is right. I have no explanation for it as yet except that it may be a coincidence that those early ones ran low. Possibly there could be something wrong with them. So far I am not willing to say there is but if I continue to get more that do not show the drop, perhaps I shall have to go to the extreme of doing them both ways to see whether I can find out what the trouble is or was. The only thing I can say now is we have had so many in which the potassium concentration does not drop that I think it should serve as a caution to us not to think that potassium is always reabsorbed completely in the proximal tubule.

DR. COOKE: Are those proximal tubules actually doing very much in regard to the handling of electrolyte? The ratios for sodium are not particularly unusual and the rise in inulin concentration is not marked.

DR. BOTT: They do very little, of course, as compared with mammalian kidneys but they are available and one can get at the various parts of the tubule. My plan was to plot the pattern first for amphibia and then go on to mammals. We all know that we cannot reach all parts of the tubules in the mammal and the knowledge gained from the general picture of the amphibian kidney might help us to figure out from fewer experiments on mammals what was actually happening. It has taken a great deal longer than I expected it to take because the results have not turned out the same for potassium in the proximal tubule. We need many more experiments and the work is very time consuming.

DR. GILMAN: Do you have any potassium figures besides those you showed?

DR. BOTT: Just those I showed. I have not any flame photometer results for mammalian tubule fluid as yet. Those in rats in one case showed a ratio higher than 1.0 for the proximal tubule when done using chemical methods.

DR. SHIPP: Do you feel that the marked variation in the tubular fluid/serum inulin ratio at any point along the proximal tubule may be referrable to a fast rate of removal of all the fluid in the tubule including that which has just been filtered? How is the net sodium and water movement influenced by the rate of flow of tubular fluid?

DR. BOTT: I can say there may be some relation to flow, yes. I cannot say it is directly correlated in all cases.

DR. LAUSON: Do you load these animals in advance with saline or other infusions?

DR. BOTT: No. So far I have not done that. I am terribly anxious to try all kinds of tricks but I have not done anything except inject a solution of inulin.

DR. LAUSON: Do you think they may be on the verge of sodium depletion at the time that you study them?

DR. BOTT: They do eat earthworms and I try to keep them well fed, but that is about all I have been able to do.

DR. LAUSON: So the fltration rates in these animals perhaps, if anything, are lower than they would be in the unanesthetized, intact animal?

DR. BOTT: I should think so.

DR. LAUSON: Perhaps they might be hypo-filtering, and that might be a contributing reason why so many of these potassium concentrations were still rather high in the proximal tubule, whereas if the nephrons were filtering adequately the result may have been different.

DR. BOTT: I doubt that because, for instance, you may have noticed in the urines of many of these animals that sodium and chloride concentrations are so low that the fluids are almost like distilled water. They must be in pretty good shape and working pretty well if that is the case.

DR. LAUSON: That is just the circumstance under which one would expect relatively more potassium to be in the urine, provided there are any anions being excreted. Of course, if all the chloride is reabsorbed, and if you are not administering sulfate or other anions, there would be very little electrolyte of any kind left in the urine.

DR. BOTT. I deliberately avoided giving any electrolyte in order to keep the picture as normal as possible but I certainly am very anxious to try all sorts of things like that, including hormones and diamox.

DR. GILMAN: I wish you would not try to persuade Dr. Bott there is anything wrong with these observations because despite the fact that I am a kalimaniac, I am completely convinved of the importance of the secretory system and the homeostasis of potassium. I am delighted to see these potassium and sodium figures come out exactly the same.

In the current issue of the AJP we have a paper in which the conclusions are based largely on indirect evidence but in the dog it appeared that there was no selective reabsorption system for potassium in the proximal tubule and that if you put the secretory system to sleep the ratio of sodium to potassium in bladder urine would be the same ratio as in plasma. From this we would like to believe that actually in the reabsorptive process, sodium and potassium are not the ionic species that are actively transported, but rather that anion is transported. In this case it would be chloride, and sodium and potassium are just reabsorbed simultaneously to maintain electroneutrality. I would like very much to believe Dr. Bott, that these are the true figures. I don't know why you want to have a selective reabsorptive system for potassium;

if cations are going back in the ratio that they are present in tubular urine, then you can see the obvious necessity for a specific transport system for potassium in the distal tubule allowing secretory transport to be the primary homeostatic mechanism in the regulation of potassium metabolism. I hope your mamalian figures come out exactly the same way.

DR. BOTT: Thank you for those kind words. I noticed your article and was very much interested in it and wanted to digest it a little more and see how I could work it in with some more of these things. If I had to say, I should say that I am getting more and more of those which do not show the potassium drop in the proximal tubule and I should not wonder if that is more the true situation.

DR. SHIPP: Dr. Homer Smith[19] suggests that the renal tubules operate primarily on sodium. He considers chloride an indifferent ion since it can be reduced to very low levels, by replacement with sulphate and nitrate, with no marked physiological disturbance if water balance is maintained. Dr. Gilman, would you please comment on this in relation to your present concept? In your recent studies how did the urinary sodium/potassium ratio compare with that in serum?

DR. GILMAN: I don't recall those figures at all but I don't see how that destroys the concept. If one introduces an anion that is not reabsorbed, how can that destroy the concept that anionic transport is primary? I would like to make one comment about the chloride humps. They also delight me because if one accepts the theory for which I believe there is a great deal of evidence, that all the bicarbonate reabsorption is the result of hydrogen ion transport and that this occurs in the proximal as well as the distal tubule. In that process one gets cationic exchange between hydrogen and sodium and a simultaneous disappearance of anion, since bicarbonate is going down to CO_2, therefore the chloride hump would be expected if chloride is being reabsorbed with equivalent amounts of fixed cation.

CHAIRMAN COOKE: We ought to see a pH change unless we make the assumption that the tubule at that point has a different pCO_2.

DR. GILMAN: There would have to be a decrease in pH.

CHAIRMAN COOKE: That was not observed. I was impressed that the chloride hump was awfully small in these animals in contrast with the rat, where I would think the pH change would have to be quite large, although you don't have these figures.

DR. BOTT: No.

CHAIRMAN COOKE: Let's see what other conclusions one could come to. If the sodium remains constant and the chloride rises very significantly, the bicarbonate must go down and the pH ought to go down considerably.

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[19] Smith, H. W., Principles of Renal Physiology, 1956 (page 96).

DR. BOTT: I think actually in the amphibian kidney that curve is so small that we were not even sure that it was real at first. I believe it is real but perhaps it is not quite as high as thought at first, at least under conditions controlled as they were in Montgomery and Pierce's experiments. It is so small that perhaps some of our changes are almost within experimental error. I am almost willing to predict that we may find some change in pH in the proximal tubule in mammals.

DR. FRED BERGLUND: What is the final pH in the Necturus urines?

DR. BOTT: Around six.

DR. BERGLUND: It does not go very low. Have you done any experiments with acid loaded animals?

DR. BOTT: I believe that Dr. Giebisch is doing some in Vienna this year.

CHAIRMAN COOKE: Dr. Taggart, would you like to comment? You have been involved.

DR. JOHN V. TAGGART: I have not been personally involved. All I can say is that I watch expectantly every result that Dr. Bott puts out.

DR. BOTT: I hope to make progress a little faster now that most of the bugs are out of the instruments and we have overcome that difficulty.

CHAIRMAN COOKE: I would like to raise a question that is possibly out of order but it is in this regard: We have the problem of the potassium wasting individual with renal disease occurring more commonly. Does this individual shed any light on the selective reabsorptive mechanism for potassium? This has been studied a great deal more in adult patients than in children. Our experience has been with only one case, a child seen by Dr. Wilkins at Johns Hopkins a little before I arrived officially. It was noted that potassium wasting continued under quite unusual circumstances. The classical concept for the excretion of potassium as of 20 minutes ago was that the bulk of the potassium or all of it was reabsorbed in the proximal tubule. Any potassium appearing in the urine was exchanged for sodium at some distal exchange point. With a larger *mass of sodium moving through the tubule (the mass factor) or some alteration in the* exchange site (the per cent factor) the amount of potassium appearing in the urine per minute is altered.

Now in the case of the child that was studied by Dr. Wilkins and his group it was striking that on a regimen in which there was essentially no sodium being administered to this individual and very little sodium appearing in the urine, there was still a significant excretion of potassium. With the administration of larger amounts of sodium there was a very great increase in the excretion of potassium, and I think that the concept that the exchange occurs and is increased by the greater mass of sodium certainly would not be ruled out in any way by those experiments. But it was impressive that with extreme sodium restriction there was still rather considerable excretion of potassium, even though the individual was quite depleted of this ion.

DR. LAUSON: That is exactly the circumstance under which you would expect to have a maximum potassium excretion, isn't it? When you force the individual to retain practically all the filtered sodium, all excreted anions must be covered by other cations.

CHAIRMAN COOKE: The anion load, which is basically chloride and phosphate was extremely low also.

DR. LAUSON: But they would be mostly neutralized by potassium.

CHAIRMAN COOKE: It was primarily potassium that was neutralizing the anions.

DR. LAUSON: The more confusing thing is when you then give the patient plenty of sodium that there should also be more potassium lost. That is harder to see.

CHAIRMAN COOKE: That is less confusing to me. It would seem to me to be fairly simple. If we have some sort of a process in which potassium is exchanged for sodium -- and I teach this to the medical students, because I don't know how to think in any more complicated fashion -- as little balls bouncing around against the wall of the exchange site. Then the more balls bouncing per minute the greater the opportunity for exchange. With large amounts of sodium moving through the tubules I would expect to see considerably more potassium being excreted simply on a basis of greater opportunity for exchange. I am not familiar with any situation in which large amounts of sodium move through the tubule except with mercurial diuresis in which we do not see markedly increased amounts of potassium.

DR. BOTT: If it turns out that the concentration of potassium remains the same as that in serum or plasma in the mammalian kidney there still might be a great deal of reabsorption of potassium in the proximal tubule because so much water is reabsorbed there: 85 per cent or so of the total. I don't know if that would be any argument that would help you explain the case that Dr. Cooke was asking about.

DR. FOX: What was the anion with the sodium administered to this patient you mentioned?

CHAIRMAN COOKE: He was put on an essentially sodium chloride free diet. When sodium was given, it was given either as sodium chloride or sodium phosphate, sodium bicarbonate produced a tremendous outpouring of potassium. This increased outpouring has been thought to be due to alkalosis. I think it is much easier to relate it to the mass of sodium that is moving rather than any pH effect.

DR. FOX: All sodium salts may not yield identical effects. Recently I have been making some studies in a man with advanced nephritis who has a urine potassium in the neighborhood of 10 and 12 mEq. per liter in a volume of not over a liter. Administering sodium has not changed the potassium excretion but the sodium was given as acetate. Sodium chloride might have a different effect. That is the point I am stressing. The role of the anion has not had sufficient attention in some of these studies. If you administer sodium chloride, potassium may leave in increased amount, whereas if you administer sodium without a fixed anion it is not necessary for potassium to come out.

CHAIRMAN COOKE: If you take a series of rats and administer similar loads of sodium, one in the form of sodium chloride and another as sodium acetate and another as sodium phosphate, the amount of potassium that comes out in the urine is dependent upon the rapidity with which the anion is excreted. When bicarbonate or acetate are given there are large amounts of potassium excreted. In the case of sodium chloride there is some delay in the excretion and less potassium comes out.

DR. FOX: May we not find species differences? Homer Smith[20] pointed out that the experiments in the dog yield different results than in man and monkey; these are also different than the rat.

CHAIRMAN COOKE: I think it has been demonstrated that the human does excrete large amounts of potassium after sodium bicarbonate loading which is essentially the same as sodium acetate loading, and considerably greater amount of potassium than when a given quantity of sodium chloride was administered.

DR. METCOFF: When non-reabsorbable anion appears in the urine it is at the expense of chloride and the chloride diminishes in the urine. There is thus induced a situation where cation excretion is augmented both for sodium and potassium and chloride excretion is diminished, suggesting there is some means whereby anion exchange can occur independently from exchange of cation.

As far as your observation on this child in Baltimore is concerned, it is interesting that even in situations where there is potassium excretion in the urine in excess of the quantities being simultaneously filtered, this is not invariably present. This occasionally appears to be associated with a reduced concentration of potassium in the muscle cell. It is conceivable a concentration change in the cell _per se_ might predispose to the secretion of potassium by the renal tubule cells.

CHAIRMAN COOKE: Actually, in terms of what we have been trying to explain, namely, the system of potassium secretion or exchange, one of the factors which is involved is intracellular composition. Another influence may be the pH of the cell. Another may be the potassium content of the renal tubular cell, although no one has been able to demonstrate its altered potassium content in any sure way. Also there are hormonal influences that affect the availability of this potassium for exchange. We did a series of experiments a few years ago in which we compared loads of sodium nitrate for example, with ammonium nitrate and there seemed to be more potassium appearing in the sodium nitrate treated animals than the ammonium treated animals. This would be a little different from what you might predict from your concept that there has to be some cation. But we were impressed that there might be an exchange of sodium for potassium fairly low down in the tubule and that if other things such as ammonia had been substituted higher up, as you might expect, with constant ammonium loading there would be less opportunity for potassium exchange.

We are five minutes behind time. Dr. Solomon, would you present your studies on transport of small molecules? Dr. Solomon!

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[20] Smith, H. W., The Kidney, Structure and Function in Health and Disease. Oxford University Press, pp. 333, 667, 1951.

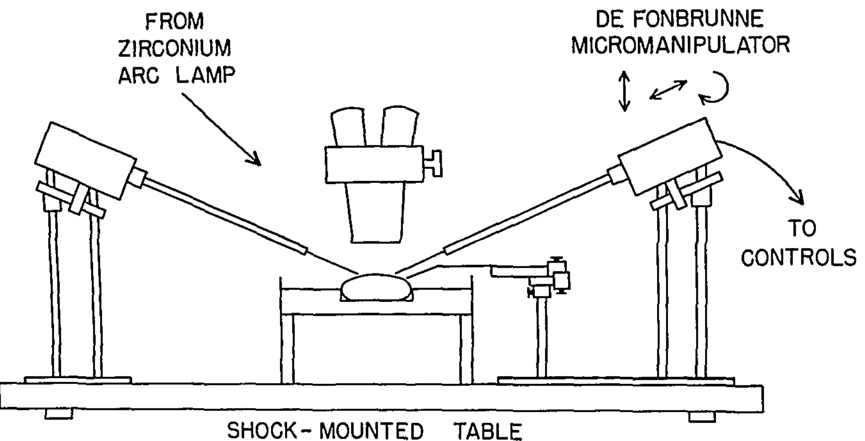


Fig. 13: Equipment for nephron micropuncture studies.

B. Studies on Perfusion of the Proximal Tubule in Necturus.

DR. ARTHUR K. SOLOMON: The results that I am presenting cover some experiments on perfusion of the proximal tubule of the kidney in Necturus. I would like to emphasize at the outset that these are the results of preliminary experiments only and that in the best of all possible worlds we would need more experiments than we now have. So please accept the following with this reservation.

The study was initiated the first of July, 1953, and has been carried out by a large group of people. From 1953 to 1955 Dr. Hanenson worked with us and developed the technique of perfusion in our laboratories. Dr. Shipp was actively engaged on the project from 1954 to 1956. Dr. Yoshimura took part in this study briefly in 1955, and Drs. Windhager and Schatzmann have been working on the problem in 1956. I hope our study can be viewed as the direct outgrowth of the classic work that has come for many

The techniques

blished in 1937.

the same issue

of the Journal. I would like to express our appreciation to Dr. Bott for her kindness in instructing Dr. Hanenson in these techniques and in helping us very generously over these past three years. Dr. Richards has also talked with us about the problem, and we are grateful for the help he has given us.

All of the experiments that we have performed have been concerned with the proximal tubule of the Necturus. In all cases the anesthetic that we used was one suggested to us by Dr. Otto Krayer: tricaine methane sulfonate [21], which goes by the code MS-222. The

ently a drop

ment of the

[22]; it was he who found the blood pressure drop of which I speak. I shall return later to some of the other results which Dr. Kinter has been kind enough to let us use in our calculations.

Let me show you in this first slide (Figure 13) the equipment which we used for these studies: a microscope with a pair of De Fonbrunne micromanipulators, standing on a shock mounted table. The controls are on the laboratory bench off the table. The oval shaped object under the microscope, in case you don't recognize it, is the Necturus. Just to the right is a rather coarse micromanipulator used to pull the capsule tight, so it can be pierced more readily with the micropipettes. There is another coarse manipulator on the left side, which has been omitted from the drawing for purposes of clarity. The illumination is provided by a Zirconium arc lamp. The De Fonbrunne micromanipulators are mounted on steel stands, which permit us to move them up and down, to rotate them about a horizontal axis, and finally to advance them along the direction of the pipette with a very rapid acting screw. The micropipettes were made of Pyrex on a Livingston pipette puller with tips of the order of 10-25 mμ in diameter. The mercury, which provided the hydraulic pressure to move the fluid in the pipette, flowed by gravity as was customary for Richards' group.

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[21] Rothlin, E., Schweiz. Med. Woch., 62:1042, 1932.

[22] Kinter, W. B., personal communication.

The experiments were of two kinds, collection and perfusion. I would like first to turn to the collection experiments. These were used primarily to determine whether our conditions of anesthesia and handling the animal provided results different from those found in the literature. Typically, I would say we collected between 0.1 and 0.2 microliters of fluid from a tubule in which an oil drop had been inserted as a block just distal to the collecting pipette.

Radioactive assay was used for inulin analysis in the collection experiments (C^{14} labeled inulin obtained from the Abbott Laboratory). In a set of 30 replications we were able to measure the inulin concentration to an accuracy of 2.8 per cent. Three studies were carried out to check that the C^{14} inulin behaved as normal unlabeled inulin. First, inulin was assayed chemically using the resorcinol method[23]. Results of the chemical analysis were identical with those obtained by C^{14} analysis. Indeed, the ratio of results obtained with the two methods is 1.00, in which the second 0 is purely fortuitous. Secondly, we injected some of the radioactive inulin into a frog, which then spent 24 hours living in a desiccator adequately supplied with oxygen. No radioactivity was found in the expired CO_2, collected over the 24-hour period. We therefore concluded that the C^{14} labeled inulin was not metabolized in the frog.

We have Dr. Kinter to thank for our third experiment. He has been doing clearance studies on Necturus using creatinine and some of our C^{14} inulin. In four experimental periods in two animals, the clearance as measured by creatinine was compared to that measured by inulin; the ratio once again contains one of these fortuitous zeros - it is 1.00 ± 0.07.

Fourteen collections have been carried out on 11 animals. Of these, 9 were in the distal half of the tubule, and gave an inulin ratio of $1.81 \pm .80$ (inulin concentration in collected fluid/inulin concentration in plasma). As you may remember, this number appears to be somewhat larger than that obtained by Dr. Bott. It is also larger than 1.23 obtained by Walker and Hudson in 16 collections in the phlorhizinized animal using glucose as an index of reabsorption. Dr. Bott, your ratios are closer to 1.3 than 1.8, aren't they?

DR. BOTT: I would have to calculate it. Certainly I have had ratios higher than 1.81. You saw them on the chart.

DR. SOLOMON: We had one figure of 3.81 in our average.

DR. BOTT: That is very high.*

DR. SOLOMON: Unfortunately, we can find no reason to throw it out. That is one of the major reasons why our average is so large. The only thing to do is to make some more collections and see what the average really is.

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* It is quite possible that higher ratios may be obtained when less inulin solution is injected. This is the very reason that inulin or some other standard should be determined simultaneously with other substances.

[23] Roe, J. H., Epstein, J. H., and Goldstein, N. P., J. Biol. Chem., 178:839, 1949.

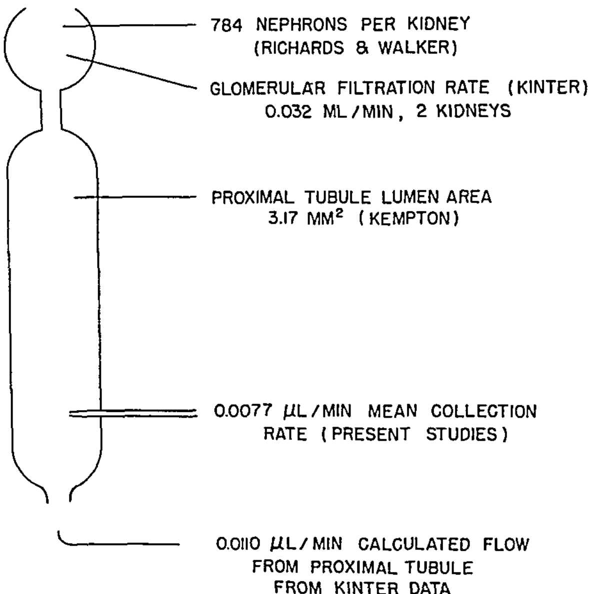


Fig. 14. Data obtained from literature.

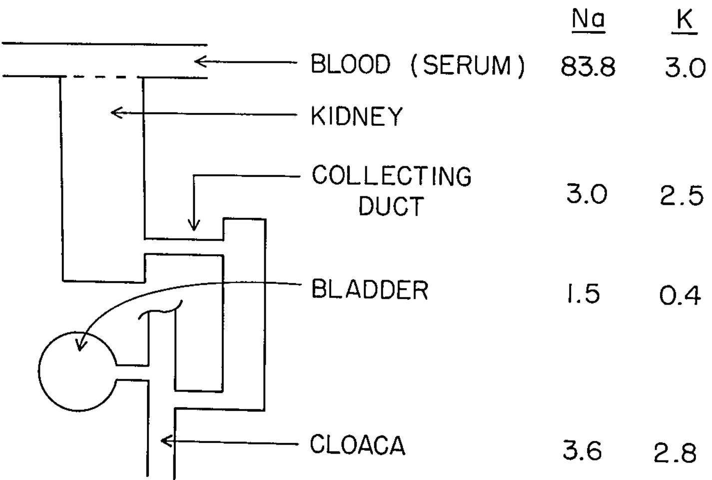


Fig. 15: Na & K concentrations in necturus urinary tract.

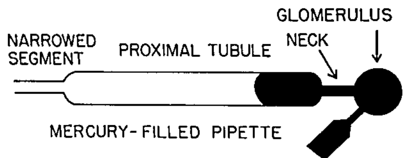
The rate of collection was also measured in some of these experiments, though not in all. In 8 collections the rate was 0.77 μl/min. I would like to show another slide (Figure 14) summarizing the various data concerning fluid flow in Necturus proximal tubules. Kinter has measured the glomerular filtration rate in pithed Necturi; in both kidneys of a pithed Necturus his measured flow is 1.9 milliliters per hour. We are responsible for all the operations that this measured number has subsequently suffered in the calculations that follow. I think Kinter's figure represents results obtained in 39 experimental periods in 16 animals. The figure of 784 nephrons per kidney is a tribute to the patience of Richards and Walker who counted all the nephrons in one kidney. Kinter's measured glomerular flow divided by the number of nephrons in both kidneys gives the glomerular filtration rate per nephron, which can be expressed in milliliters per minute per nephron. From our presently observed inulin ratio of 1.81 (collected fluid/serum) and Kinter's observed glomerular filtration rate, it can be calculated that at the end of the proximal tubule 0.011 microliters of fluid per minute will be discharged. Our collection rate in the same studies from which the 1.81 ratio was obtained -- at least some are from the same series -- was 0.0077 μl/min. We take the agreement between these two numbers as an indication that the kidneys were behaving in the present experiments in a more or less normal fashion.

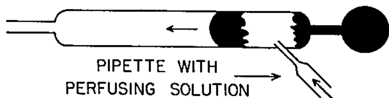
Alternatively, the same calculation can be done backwards. If instead of looking at the (distal) end of the proximal tubule you look at what goes through the glomerulus you can take the data of Walker and Hudson, their value of 1.23, and their mean collection rate which is 0.0145 μl/min. From these figures a glomerular filtration rate of 0.0178 μl/min. can be calculated for Walker and Hudson's experiments. The present studies give a glomerular filtration rate of 0.014 μl/min. per nephron. Kinter's data lead to a value of 0.0205 μl/min. which is slightly larger than either of these. It appears that the animal is not unduly harmed under our conditions of operation.

In order to reassure ourselves on this point, one other measurement was made on collected fluid. The results are given in Figure 15. Sodium and potassium concentrations were measured in different portions of the Necturus' urinary tract. For 3 animals, the average for sodium in the serum was 83.8 meq./l; in the collecting duct it was 3.0 meq./l fluid; in the bladder it was 1.5 meq./l fluid and for the cloaca 3.6 meq./l fluid. These figures show that the animals under our conditions of treatment are capable of putting out a urine which is very dilute as far as sodium is concerned.

Now let me turn to the perfusion studies. Figure 16 shows schematically the techniques used for the kidney tubule perfusions, following the method described by Richards' group. A mercury filled pipette is inserted into the glomerulus. The glomerulus is filled with mercury and enough pressure is exerted to force the mercury through the neck into the proximal segment of the proximal tubule. This pipette is replaced with another one containing perfusing solution, which is then inserted into the mercury drop at the proximal end of the tubule. The drop is then split and one end pushed down the tubule to stop, hopefully, at the junction with the thin segment.

The solution used for perfusion contains sodium, potassium, chloride, phosphate, calcium and bicarbonate. The sodium concentration of about 97.5 meq./l serum was chosen on the basis of analyses done by Dr. Yoshimura in May and June, 1955. The





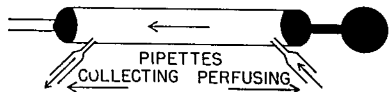


Fig. 16: Schema of technique for nephron perfusion.

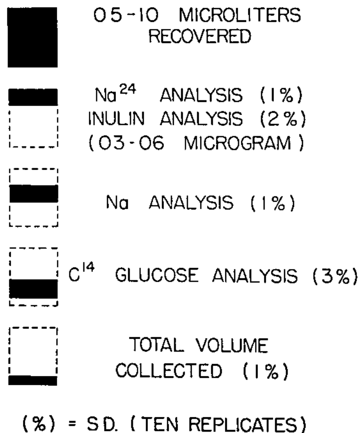


Fig. 17: Analysis of fluid collected from single perfused renal tubule.

results of his analyses on 9 animals gave a sodium concentration of 97.5 ± 4.6 meq/l serum. This large standard deviation is characteristic of our experience with the animal. Subsequently, in July, 1955, Dr. Yoshimura carried out another set of analyses for serum sodium in a series of 5 animals and obtained a value of 84.8 ± 2.0 meq/l serum. Consequently, we think there may be a seasonal variation in the serum sodium concentration in this particular animal.

Hopefully, in the course of a perfusion we would obtain somewhere between a half and 1 μl of fluid over a perfusion period of 30 minutes to 1 hour; this material was analyzed for a great many substances. Figure 17 shows the kinds of analysis made; the figures in parentheses give the standard deviation on the basis of 10 replicates of samples of similar sizes. Na^{24} gamma radiation was measured in a scintillation counter, which could easily be done to one per cent. Inulin was measured to about 2 per cent by the Roe, Epstein, and Goldstein[23] resorcinol method as modified by Lowry[24] for microanalysis. Total sodium was measured on a Beckman flame photometer as modified by Solomon and Caton[25]. In samples of this magnitude it gave an accuracy of one per cent. C^{14} glucose, which was occasionally used, was measured in a Robinson flow counter[26] to an accuracy of about 3 per cent. Finally, the total volume of fluid could be measured by collecting all the residual fluid and measuring its volume from its radioactivity, which could be done to one per cent. We identified the length of perfused tubule in as many cases as we could, using the neoprene latex method developed by Bott[16].

Let me turn now to the results obtained with C^{14} glucose, and its disappearance in the perfused proximal tubule. In 6 experiments the relative C^{14} glucose leaving the tubule was 35 per cent of what went in, expressed in counts per minute per milliliter. There was a small water shift which would raise this figure to 39 per cent. Using the inulin analyses --

DR. LAUSON: Down to 35 per cent of the original?

DR. SOLOMON: We took out 65 per cent. The C^{14} glucose concentration is down to 35 per cent of its initial value. This was a very encouraging finding because it compares favorably with the results obtained by Walker and Hudson.

CHAIRMAN COOKE: Over how long a time did this occur -- the time between sampling, or was this just continuous flow?

DR. SOLOMON. How do you mean?

CHAIRMAN COOKE: You put in fluid and take it out?

DR. SOLOMON: We take it out from the distal end of the proximal tubule. It takes about half an hour to one hour to collect a sample large enough to count. The average volume of the tubule, from Kempton's figures[27], is 5.5×10^{-2} cubic milli-

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[24] Lowry, O. H., personal communication.
[25] Solomon, A. K., and Caton, D. C., Anal. Chem., 27:1849, 1955.
[26] Robinson, C. V., Rev. Sci. Inst., 22:353, 1951.
[27] Kempton, R. T., J. Morph., 61:51, 1937.

meters. Since roughly it takes half an hour to collect half a μl, it takes about 3 minutes for a slug of fluid to traverse the proximal tubule.

In a tremendous series of 42 observations, Walker and Hudson found that 55 per cent of the glucose was reabsorbed. Their mean velocity of perfusion was 0.019 μl/min. This figure is not given in their paper, and I have taken the liberty of assuming the rate was the same as that given in an adjacent table (their table 4), from which I have calculated the mean velocity of perfusion. Our comparable figures are 65 per cent reabsorbed with a mean velocity of 0.020 μl/min.

The agreement in these two sets of observations was very welcome to us on another score, because Walker and Hudson measured total glucose in the collected fluid and we measured C^{14} glucose. We were measuring, if you will, the efflux of C^{14} glucose from the tubule. They were measuring the efflux minus the influx because any glucose that entered the tubule contributed to their measured value. Since we have not been able to develop a good chemical glucose method for measurements in fluid collected from the tubule, we had no way of estimating how much glucose entered the tubule from the blood. However, the very fact that the present results agree so well with those of Walker and Hudson leads us to conclude that the amount of back transport of glucose from the plasma into the tubule must be relatively small.

TABLE 6

Glucose Efflux From Proximal Tubule of Necturus

Exp.	$\frac{(v)}{(t)}$ μl/min.	C^{14} Glucose (Cpm/μl) Perfused	Collected	Glucose Efflux $\mu\mu$M/min. tubule
23	0.008	225	15.9	15.4
24	0.015	355	0	---
18	0.016	324	188	27.2
25	0.019	281	141	17.4
16	0.031	447	247	59.3
20	0.032	242	100	32.5
22	0.217	214	206	22.5
			Average	29.1

On that basis we have calculated the efflux of glucose from the proximal tubule (Table 6). In order to make this calculation it is necessary to make the following assumptions: one, that the influx from the blood to the tubule is negligible; and two, that the efflux is proportional to the glucose concentration within the tubule. In the second column you see the velocity of perfusion, with the experiments entered in the order of increasing velocity. In the next column, the raw data is given, in counts per minute per μl of fluid collected. In experiment 23, at the top, which represents the slowest perfusion we have ever achieved, almost all the glucose is removed. In experiment 24, which is also slow, there was no glucose that we could measure in the collected perfusate. The velocity of perfusion was inadvertently far too high in experiment 22, and there was almost no difference in the glucose concentration between perfused

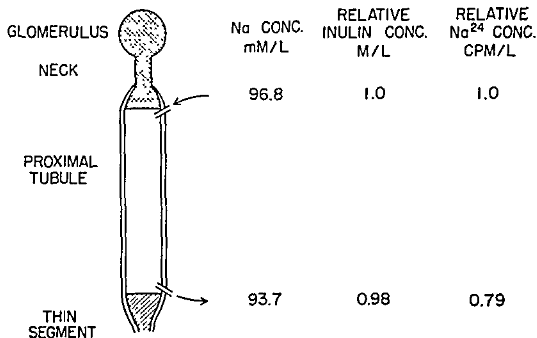


Fig. 18 Sodium and water shifts in necturus proximal tubule (11 perfusions)

and collected fluids. Nonetheless when the glucose efflux is calculated in terms of rate, expressed in micromicromols per minute tubule (fifth column), no such monotopic progression is evident. The efflux rate is more or less constant, which is to be expected. Thus, though there was a very much larger removal of glucose from fluid that spent only a few minutes in the tubule, this larger number must be divided by a larger number of minutes to obtain the rate. Glucose efflux is not calculated for experiment 24, because I have not yet thought of a nice approximation for dealing with that zero in the fourth column which makes everything go to infinity.

Next I would like to turn to the results that have been obtained in our measurements of sodium and water flow out of the tubule. Figure 18 shows these results expressed in graphical fashion. In 11 perfusions the mean sodium concentration entering was 96.8 meq. l; the mean effluent concentration was 93.6 meq. Na/l. The mean inulin concentration showed no difference at all, and the relative sodium^{24} concentration fell. Since inulin and sodium concentrations are more or less unchanged while the Na^{24} concentration has become smaller, sodium must have moved in both directions across the tubular membrane.

The disturbing fact in this set of observations is the absence of the expected increase in inulin concentration. It was indeed this observation that led us to undertake collections ourselves since Walker and Hudson, and Bott had both shown water reabsorption in collection experiments in the Necturus.

We hoped to determine whethere there was something wrong with our whole schema or whether the discrepancy could be reduced to the simple process (perhaps "simple" is an overstatement) -- to the laborious process of perfusion. There is evidence to support the belief that sodium efflux from tubules may not work as well in a perfused animal as in a normal animal. Thus, Swanson[28], who has made measurements on the doubly perfused bullfrog kidney, finds in the non-perfused kidneys that the ureteral urine has 5 to 15 per cent of the sodium concentration of the plasma; this may be compared to his results in the perfused bullfrog in which the urine sodium concentration has risen to 50 per cent of that in the perfusing fluid. This observation, coupled with the results of our collection experiments, leads us to believe that the kidney tubule is not being damaged in an irretrievable fashion under our conditions of anesthesia and operation, and that the discrepancy may be ascribed to the differences between the conditions of perfusion and those of collection.

To obtain quantitative results from the present data, it is necessary to go through a rather elaborate mathematical treatment. This arises because everything in the tubule changes during an experiment and nothing is in the steady state. First, the sodium concentration changes. Secondly, the Na^{24} concentration in the tubule changes as Na^{24} is transported out of the tubule. Thirdly, though on the average in 11 experiments water did not move across the tubules, in many of the individual cases the water did move. Three equations have been developed to take account of all these features, in a quantitative fashion. I would like to show you these equations, not to emphasize the equations themselves, but because the assumptions used for each of these equations

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[28] Swanson, R. E., Am. J. Physiol., 184:527, 1956.

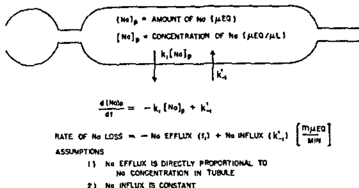


Fig. 19: Equation describing movement of inorganic sodium across the tubule.

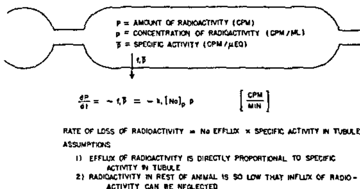


Fig. 20: Flow of radioactivity across tubule.

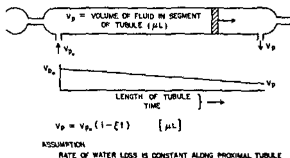


Fig. 21: Flow of water across tubule.

Na FLUX CALCULATIONS FOR KIDNEY TUBULE

$$\text{NET EFFLUX} = \left(\frac{V}{t}\right)_{OBS}\left(\frac{[In]}{[In]_o}[Na]_{p_o} - [Na]_p\right)$$

$$\text{INFLUX} = \left(\frac{V}{t}\right)_{OBS}\left(\frac{[In]}{[In]_o} - 1\right)\frac{\mathrm{Ln}\; p_o/p}{\mathrm{Ln}\,[In]_o/[In]}\left(\frac{\frac{p}{p_o}[Na]_{p_o} - [Na]_p}{1 - \frac{p}{p_o}}\right)$$

EFFLUX = INFLUX + NET EFFLUX

Fig. 22 Calculations of sodium flux in kidney tubules.

are essential to the calculations. If the assumptions are wrong, then everything that follows is wrong.

The first equation (Figure 19) concerns the movement of inorganic sodium across the tubule. The equation says that the rate of sodium loss from the tubule is given by the difference between sodium efflux and sodium influx. Further, it says that the sodium efflux is directly proportional to the sodium concentration in the tubule. That is the first assumption. The second assumption states that the sodium influx is constant. This assumption seems plausible because no perturbation is applied to the plasma, which comprises the extratubular environment of the system. In sum, the basic assumptions are first, that the sodium efflux is proportional to the concentration in the tubule; and, second, that the sodium influx is constant.

Figure 20 shows the equation describing the flow of radioactivity. This equation says that the rate of loss of radioactivity is given by the sodium efflux times the specific activity in the tubule; that is, the rate at which sodium moves out times the fraction of sodium molecules inside the tubule that are labeled. For this equation, the first assumption is that the efflux of radioactivity is directly proportional to the specific activity in the tubule, and the second assumption is that the radioactivity in the rest of the animal is so low that there is no return flux of radioactivity back into the tubule.

The third and final one of these equations is shown in Figure 21. Here we are dealing with water only. I have shown the water flow in the direction of reabsorption, but formally, it does not matter which way it goes. The assumption that we are making is that the rate of water loss is constant along the proximal tubule, and that the volume of an entering slug of fluid follows in a straight line course from the time the slug enters until the time that it leaves. The subscript zero is used here and later to denote the initial value of a variable.

Starting with these three equations, two pages of arithmetic will lead to the results shown in Figure 22. The exact form of the equations shown is really quite unimportant, but it is important that every number shown on the right hand side of the equation be directly measurable. The net efflux is, of course, the difference between the amount of sodium in the slug which entered the tubule and the amount in the slug which is withdrawn from the tubule, after correction for water shift. This is given in terms of the observed rate of perfusion [volume collected/time of perfusion, (v/t)obs], the inulin concentration ratio, and the initial and final sodium concentrations. The influx is made up of exactly the same components with the addition of the initial and final radioactivities. All the variables on the right hand side of the top two equations are directly measurable, the inulin and radioactivity ratios, the rate of perfusion, and the initial and final sodium concentrations.

Let us now look at the results which have been obtained in these perfusion experiments. Table 7 shows 7 experiments that are as normal as we know how to make them. The perfusion rate was 0.027 μl/min. Note the large standard deviation in the fluxes. Everything I say from this point on must be colored by this large standard deviation. Indeed, most of the numerical differences that will be discussed are of no statistical validity. They are only indications, so please accept them at your peril.

One indication is that the efflux of sodium may be a little greater than the influx. In these 7 experiments, the efflux was greater than the influx in 4, and it was less in 3. One might be tempted to say that the sodium is just running down its concentration

TABLE 7

Relation Between Rate of Perfusion and Sodium Flux in Necturus Proximal Tubule

	Na flux Influx (mμeq/min. tubule)	Na flux Efflux (mμeq/min. tubule)
Normal (7 experiments) perfusion rate 0.027 ± 0.005 μl/min.	0.69 ± 0.33	0.74 ± 0.50
Fast perfusion (6 experiments) perfusion rate 0.099 ± 0.042 μl/min.	1.81 ± 0.76	1.90 ± 0.76

gradient. To show you why one might be tempted, let us examine the sodium concentration in the perfusion fluid, which was initially selected on the basis of Yoshimura's analysis of Necturus in May and June. Since these animals had a serum sodium concentration of 97.5 meq/l serum, the perfusion fluid was made up to approximate this value; actually it came out to be 100.5 meq/l fluid on the average, due in part to the addition of radioactive Na^{24}. The final sodium concentration in the collected perfusate in these experiments came out to be 96.3 meq/l. Unfortunately, we did not measure the concentration of sodium in the serum of the animals actually perfused. In order to obtain an estimate of this serum sodium concentration, we took advantage of a subsequent observation, which agrees with the observations made by Dr. Bott, namely, that the sodium concentration in the latter half of the proximal tubule is exactly the same as the sodium concentration in the plasma. In a set of 7 experiments this measured ratio came out to be 1.02 ± 0.12. Once again, one of our character .tic observations: the number is 1 for a large series, but the standard deviation is so large that you cannot be sure about this 1. However, it is reasonable to assum^ ^n this basis that the initial sodium in the perfusate was more concentrated than the sodium in the plasma in this set of normals.

Nonetheless, we are not inclined to believe that the efflux of sodium from the tubule represents downhill movement, since measurements have been made which show that the potential within the tubule is negative to that in the plasma. Dr. Giebisch[29] has been kind enough to inform us of some of his unpublished results -- he finds as his lowest value that the tubule is 10 millivolts negative to the plasma. Dr. Sidney Solomon[30] finds the tubule to be even more negative to the plasma. These potential measurements were made in other laboratories on tubules which were not being perfused, and a certain risk is involved in extrapolating the results to tubules in which perfusions were carried out. It is not impossible that the absence of water reabsorption in our perfused tubules may be ascribed to an abolition of potential difference between

[29] Giebisch, G., personal communication.
[30] Solomon, S., Fed. Proc., 15:174, 1956.

tubule contents and plasma. However, the potential difference is characteristic of living tissue, so I hope that this would not be the case. Furthermore, the tubules did absorb glucose at the normal rate.

If the inside of the tubule is 10 millivolts negative to the outside, then the sodium going from inside to the outside must climb this potential gradient. The total electrochemical gradient, expressed in concentration units, is about 1.5. That is, a 10 millivolt potential gradient is equivalent to a 50 per cent increase in sodium concentration in the plasma. Thus, on the basis of this argument, sodium efflux from the tubule would not be a passive process if the influx and efflux are approximately equal in magnitude, unless it be purely an exchange progess.

Table 7 also illustrates one of our more puzzling observations. Before becoming very skillful, we did our experiments at faster perfusion rates than we did later on. Six of the early experiments were carried out at a perfusion rate which is, on the average, almost 4 times greater than those we call normal. As already indicated in the glucose studies, the influx and efflux expressed in meq/min. should be insensitive to changes in perfusion rate, since the fluxes are themselves rates. However, this is not the case. The fluxes at the faster perfusion rates are appreciably higher, though not statistically higher, than the values which we have chosen to call normal. We have not yet been able to think of any reason to account for this difference.

DR. LAUSON: Are the tubules more distended when you perfuse 4 times as fast?

DR. SOLOMON: This is what I tried to sell my colleagues who were actually doing the perfusions, but they said, "Nonsense."

DR. LAUSON: If not, why?

DR. SOLOMON: It would be fine if that were so.

DR. LAUSON: Am I right?

DR. SHIPP: The rate of perfusion, or more properly the rate of collection, was faster in those particular experiments, of the order of 0.04 to 0.05 cu. mm. per minute. Infusion and collection was controlled by manual movement of mercury reservoirs on the inflow and outflow sides and it is difficult to maintain a uniformly slow rate of flow. The mean rate of flow is calculated from the total volume collected and the duration of the perfusion. If there are periods of rapid perfusion mixed with intervals of slow flow this mean rate may be misleading.

DR. SOLOMON: The mean is the average over the time which we have collected. Because the flux is a mean rate, it has units of mμeq/min. Changes in the rate during an experiment should not affect the calculated fluxes.

DR. OLIVER: In the old fashioned perfusion experiments of frog kidneys it was easy to demonstrate that if you simply increased the arterial pressure, output of urine increased, sugar appeared in the urine and the concentration of electrolyte rose; lower the pressure and rate of arterial flow and the volume of urine dropped and the sugar

disappeared and the concentrations of salts fell[31].

DR. SOLOMON: We agree with this too, but if the calculations are made in units of mμeq/min., that should take account of it. The fluxes, which are rates, should be independent of flow rate because if the slug had gone so fast that it spent only a small fraction of time in the tubule, then only a small amount of sodium would be absorbed, even though it is absorbed at the same rate; whereas, if the slug is slowed down so that it just walks along quietly through the tubule, it spends a long time there and more sodium is absorbed. However, in both cases, absorption would theoretically take place at the same rate, expressed in mμeq/min.

CHAIRMAN COOKE: I don't see how fluid flows faster along the tubule when there is a fixed resistance which depends upon the cross-sectional area of the tubule, unless you could increase the perfusion pressure. So pressure must be higher in the more rapid perfusion.

DR. SOLOMON: The tips of the two pipettes offer the major resistance to flow, *since it is the tips that are 10 to 25 mμ in diameter. It is not the tubule. The tubule is* a great big fat 50 mμ.

DR. LANGE: You use pipettes of a different diameter for different speeds?

DR. SOLOMON: It wasn't this. It is really very difficult indeed to get slow perfusions. Dr. Shipp can explain that.

DR. SHIPP: This brings out one of the major obstacles which is constant plugging of the tip of the pipettes. This is especially true on the outflow side when perfusing at a slow rate. Often one can see particulate matter on the pipette tip which by partial occlusion makes it difficult to maintain a uniform rate of flow. Complete blockage is evident promptly by distention of the tubule or by leakage of fluid around the pipettes. There was no apparent tubule distention or leakage in the experiments discussed by Dr. Solomon.

DR. FOX. If you apply Bernoulli's principle as the velocity increases the pressure will decrease --

CHAIRMAN COOKE: For you to get a higher velocity you have to apply a higher pressure.

DR. SOLOMON: The pressure drop is not across the tubule. The pressure drop is across the tip of the pipette. Let me go on with the rest of my story, if I may.

It has been frequently suggested that the absence of reabsorption in these tubules may be ascribed to the two drops of mercury at opposite ends of the tubule. Certainly, it is a well-known fact that mercury interferes with the normal function of the kidney. Consequently, we spent a good deal of time examining the effect of mercury on the tubules. I was, I must confess, somewhat shocked to learn that metallic mercury is

[31] Oliver, J. and Shenky, E., J. Exp. Med., 53:763, 1931.

soluble in water. We decided that the only way to determine the amount of mercury that dissolved during passage of the perfusate through the tubule, was to measure it experimentally. To do this, we procured radioactive mercury^{203} and carried out experiments using radioactive mercury and a non-radioactive perfusate. In the 2 experiments performed, we calculated that the mercury concentration in the collected perfusate was 4×10^{-5} mols per liter. This is a surprisingly high concentration, although it is a very small amount of mercury to measure. Since the volume available for measurement was slightly more than 10^{-7} liters, we measured only about 10^{-12} mols of mercury.

DR. LAUSON: This neglects the fact that the cells in between may have picked up a lot of the mercury.

DR. SOLOMON: You are getting ahead of the argument. Thanks to Giebisch and Dorman[32], the tissue/plasma concentration ratio for mercury has been determined in the Necturus. In a set of 3 experiments done with measurements up to three hours after injection of Hg^{203} labeled neohydrin, they find an average tissue/plasma ratio of 1.4. On the basis of this, the T/P ratio, obtained not with bichloride of mercury, but with neohydrin, we can calculate that under our conditions the concentration of mercury in tissue would be 12 gamma per gram of tissue. We are also grateful to Dr. Giebisch [29] for having made the other measurements which make it possible to assess the importance of this concentration. In a series of unpublished results, he measured the effect of mercury on the potential difference between tubule and plasma; he found no effect in concentrations up to 100 gamma per gram of tissue. This result would suggest that at a concentration of 12 gamma per gram of tissue, we might expect no effect, provided that the mechanism associated with the development of the potential is also the mechanism which is associated with the transfer of sodium across the tubule.

DR. RAPOPORT: Can you use his plasma values against your perfusate values? As I remember, any mercurial in plasma is not all in solution. A good bit of it is carried bound to plasma protein.

DR. SOLOMON: That I think would only make it worse for us, would it not?

DR. RAPOPORT: Yes.

DR. SOLOMON: We have a factor which makes it better for us. In experiments done in dogs, 160 minutes after injection, Kessler, Lazano, and Pitts[33] have measured the T/P ratio for bichloride of mercury as compared with the T/P ratio for neohydrin. Their distribution ratio for bichloride of mercury is 0.34 times that for neohydrin. We would think, therefore, that the tissue would contain less than the 12 gamma of mercury calculated on the basis of the neohydrin distribution.

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[32] Giebisch, G., and Dorman, P. J., personal communication.
[33] Kessler, R., Lazano, R., and Pitts, R. F., personal communication.

It is also possible to make some direct measurements. Table 8 shows the results obtained in two experiments in which the fluxes in tubules are compared with those in tubules containing excess mercury. In the normal, the mercury concentration as measured by Hg^{203} was 3×10^{-5} mols of mercury/l of perfusate. Using bichloride of mercury, the concentration is raised to 2×10^{-3} mols of mercury per liter. The sodium fluxes apparently remain in the normal ranges.

TABLE 8

Effect of Mercury on Sodium Flux in Necturus Proximal Tubule

	Na flux Influx (mμeq/min. tubule)	Na flux Efflux (mμeq/min. tubule)
Normal (7 experiments)	0.69 ± 0.33	0.74 ± 0.50
Mercury only in perfusate (2 experiments) $HgCl_2$, 2×10^{-3} M/l	0.51 ± 0.10	0.66 ± 0.18
Mercury in whole animal (2 experiments) Thiomerin, 4×10^{-4} M Hg/kg	1.79 ± 0.18	2.01 ± 0.78

DR. GILMAN: *Your definition of normal tubule is a tubule of 4×10^{-5}, up to this?*

DR. SOLOMON: No further change is expected. We chose a concentration of 2×10^{-3} mols/l so that we would produce a maximal effect. Mudge[34] had observed effects on potassium flux in rabbit cortex slices with concentrations of 6×10^{-4} and 3×10^{-3} mols of mercury per liter of medium. Furthermore, Richards[35] found that he could inhibit water reabsorption in the frog using 4×10^{-5} mols of mercury per liter, provided that he injected the bichloride of mercury both into the tubules and into the capillary circulation of the animal. Why he did this, I don't know, but he very clearly states that the mercury was on both sides of the tubule.

We next decided to see what results we would get by putting a large dose of mercury in the whole animal. Thiomerin, in a dose of 4×10^{-4} mols of mercury per kilo of animal, appears to raise the fluxes in both directions. The change is so large that the effect seems to be real. However, the small standard deviation in the fluxes is actually misleading since there are only 2 experiments involved. One cannot be at all sure that the results of this small sample would hold for 5 or 10 experiments. Nevertheless, if these 2 experiments are representative, one can affect the flux by treating the whole animal with very large doses of mercury.

[34] Mudge, G. H., Am. J. Physiol., 173:511, 1953.
[35] Richards, A. N., Trans. Assoc. Am. Physicians, 44:64, 1929.

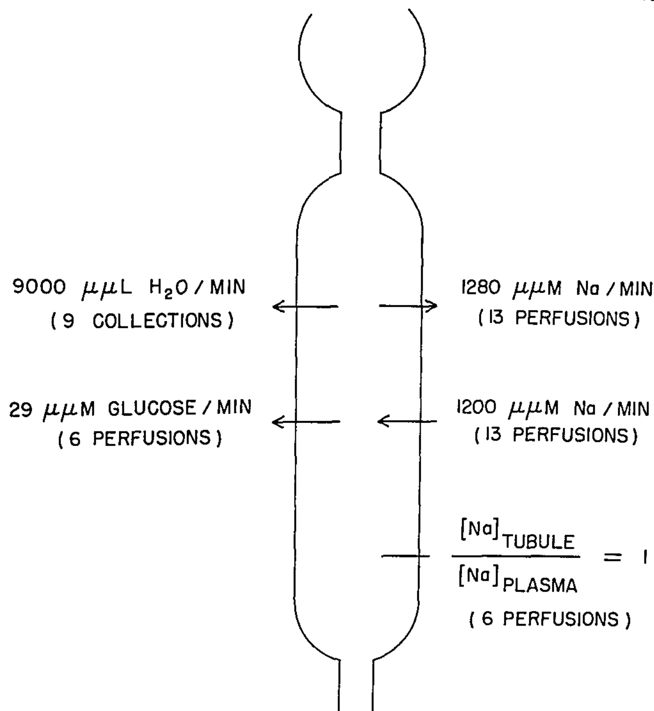


Fig. 23: Some observations on transport across the proximal tubule of the necturus.

Our conclusions on the effect of mercury on sodium transport in the proximal tubule of the Necturus are, first: excessive doses of mercury in the whole animal increase sodium flux. Second, if mercury in a concentration of 2×10^{-3} mols per liter is added to the perfusate only, it does not appear to affect normal sodium flux. This measurement, coupled with the results of Giebisch's work, lead us to believe that no effect is to be expected from the concentration level of mercury that obtains in our normal animal.

We did one other set of experiments which show the effect of tubular sodium concentration on sodium flux (Table 9). Here, the same set of normals which have been used in the previous cases, are compared with results obtained with hypertonic perfusate in which the mean sodium concentration was 115.8 ± 17.6 meq./l. Bearing in mind the limits imposed by our statistical standard deviation, there appears to be an appreciable increase in sodium efflux from the tubule, as would be expected from the increased sodium concentration inside the tubule. An increased concentration gradient should result in increased flux, on either a chemical or a physical basis. There is a very much smaller effect on the influx than on the efflux. This is to be expected, if the influx is independent of the sodium concentration inside the tubule.

TABLE 9

Relation Between Tubular Sodium Concentration and Sodium Flux in Necturus Proximal Tubules

	Na flux (mμeq/min. tubule)	
	Influx	Efflux
Normal (7 experiments) sodium concentration 98.5 ± 4.2 meq./l	0.69 ± 0.33	0.74 ± 0.50
Hypertonic (5 experiments) sodium concentration 115.8 ± 17.6	1.06 ± 0.74	1.62 ± 0.79

I would now like to summarize the results which have been obtained in these preliminary studies in Figure 23. All the fluxes, except for the water flux, are expressed in the same units. First, I would like to call your attention to the difference in order of magnitude between the sodium fluxes and glucose flux. The glucose efflux is 29 micromicromols per minute tubule and the sodium efflux is 1280 micromols of sodium per minute tubule. The water efflux, calculated from the results obtained in 9 collections in which the average inulin urine/plasma ratio was 1.81 is 9,000 micromicroliters of water per minute tubule. The ratio of the sodium concentration in the fluid emerging from the tubule to the sodium concentration in the plasma is 1, as measured in 6 perfusions.

It seems instructive to calculate the change required in the sodium flux in order to pump this much water out by a sodium pump. The calculation has been made on the basis that 90 meq. of sodium chloride moving across the tubular membrane will pump 1 liter of water across the membrane. This would require an increase in efflux of only

about 65 per cent, which would mean an additional 810 micromicromols of sodium per minute tubule. The water flow measurements were based on collection experiments. The rest of these calculations are based on perfusion data. If we could make the sodium efflux mechanism work 65 per cent better in the perfusions, we could pump the necessary volume of water. If it turns out, as I imagine it will when we do more collections, that our reabsorption (in the collection experiments) will approach the results obtained in the massive amount of data collected by Drs. Bott and Walker and Hudson, then a smaller increase in sodium efflux will suffice to pump water.

DR. LAUSON: For clarity may I ask here whether the perfusion experiments were separate from the collection experiments?

DR. SOLOMON: Yes. Table 10 presents a comparison of the fluxes of sodium that have been obtained in the present experiments with other fluxes which can be found in the literature. The units have been changed to micromicromols per square centimeter second. The values of Kempton have been used to calculate the area inside the tubule. The length of perfused tubule in 8 or 9 of our experiments has been measured by the neoprene method of Dr. Bott. The Sepia axon has a resting flux of 61 $\mu\mu M/cm^2 sec.$, according to Keynes[36]. During activity the influx goes up to 1100 $\mu\mu M/cm^2 sec.$ The sodium flux across the frog stomach, which is presumably a passive process, had a value of 200 $\mu\mu M/cm^2 sec.$, as measured by Heinz and Durbin[37] and others. The frog skin, which moves sodium actively, has a flux of 500 $\mu\mu M/cm^2 sec.$[38]. The rat intestine, which also presumably moves sodium across actively, has a flux of 1600 $\mu\mu M/cm^2 sec.$, from the results of some unpublished experiments of Mr. Curran and myself.

TABLE 10

Some Comparative Na Fluxes

Tissue	Flux $\mu\mu M/cm^2 sec.$		
Sepia Axon	61	resting influx	Keynes
	1100	influx during activity	
Frog Stomach	200	passive	Heinz and Durbin
Frog Skin	500	active	Ussing and Zerahn
Rat Intestine	1600	efflux-presumably active	Curran and Solomon
Guinea Pig Kidney Cortex Slice	380	flux into tissue	Whittam and Davies
Necturus Proximal Tubule	1200	efflux from tubule	Present studies

[36] Keynes, R. D., J. Physiol., 113:73, 1951.
[37] Heinz, E., and Durbin, R. P., Fed. Proc., 15:272, 1956, and personal communication.
[38] Ussing, H. H., and Zerahn, K., Acta Physiol. Scand. 23:110, 1951.

It is interesting to calculate the sodium flux in guinea pig kidney cortex slices from the uptake studies of Whittam and Davies[39], combined with the histological data, summarized by von Möllendorff[40]. This leads to a calculated flux of 380 $\mu\mu M/cm^2 sec$. In the proximal tubules in the Necturi we have obtained an efflux of 1200 $\mu\mu M/cm^2 sec$. This bears a close relation to the flux of sodium across the tubule of man which has been calculated by Ussing [41] from Rehberg's chloride work of the mid twenties[42]. According to this data, the flux in man is 3,000 $\mu\mu M/cm^2 sec$.

Thank you!

DR. LAUSON: If you increase the outflux of sodium to account for the outward movement of water to make the water reabsorption system in the proximal tubule fully normal, do you predict that then there would be no change in the influx of sodium and therefore no tendency for water to move right back in again?

DR. SOLOMON: Yes. As I said before, of that set of 7 normals, 4 showed a net movement out and 3 showed a net movement in. The two fluxes are the almost the same. Theoretically you can alter whichever you would like. For example, you can cut the influx down. It seems to me that the influx probably reflects what is happening outside whereas the efflux reflects what is happening inside the tubule. The mechanism which is affected by our manipulation is probably the tubule itself. It would seem more likely, therefore, to expect that the normal situation would be an increase in efflux from the tubule, rather than a decrease in the influx.

DR. FOX: I think Dr. Solomon should be congratulated on a very beautiful study.

DR. SOLOMON: The congratulation on the study, let me hasten to say, belongs to a large group of people. It belongs to Richards, Walker, Hudson, Bott, Hanenson, Shipp, Windhager and Schatzmann.

CHAIRMAN COOKE: Is there any other discussion? I think the audience may be in the same state of coma after some of the data as I am and I really cannot say anything other than I admire the work tremendously. It would be awfully nice to have a year to think it over.

DR. OLIVER: I would like to ask one question. Did I understand you made some calculations on man in which you used physical data as to the size of the nephron?

DR. SOLOMON: I was lucky. I would never have dared to do it. Ussing did it.

[39] Whittam, R., and Davies, R. E., Biochem. J., 56:445, 1954.

[40] v. Möllendorff, W., Handbuch der Mikroskopischen Anatomie des Menschen, ed. 7, Part 1, p. 24, Berlin, Springer, 1930.

[41] Ussing, H. H., in Ion Transport across Membranes, Hans Clark, Ed., New York, Academic Press, p. 3, 1954.

[42] Rehberg, P. B., Biochem. J., 20, 461, 1926.

DR. OLIVER: Where did he get the data? That is something I would be very much interested in knowing because to my knowledge after a careful search of the literature there have been only 14 proximal convolutions measured by two investigators from 4 kidneys, the weight and size of which were unstated. The figures given by the two investigators vary by over 20 per cent.

DR. SOLOMON: Ussing just gives a figure in his table, with a reference to Rehberg. As far as I remember Rehberg gives no data except the chloride fluxes, from which Ussing could calculate the sodium flux. I was delighted to see this calculation which came to my notice after the calculations on our experiment were complete. It is not included in Table 9.

DR. OLIVER: I am not sure about the bullfrog but I am fairly certain that in mammals no one has more than a vague guess, even as to the size or diameter of any physical measurement of even the proximal convolution, much less the remainder of the nephron. One of the current projects in my laboratory is to try to obtain statistically valid data on the size and number of the nephrons in those animals which are frequently used for functional renal studies, such as dog, rat, rabbit and man. It is obviously hazardous for a functional investigator to base his inferences on the currently available data. I have discussed this problem in the recent Livre Jubilaire in honor of Prof. Govaerts and I think it would be much safer for functional correlation if things were counted and measured rather than "calculated".

DR. SOLOMON: There is one question I would like to ask Dr. Oliver. One number that belongs in Table 9 is left out; that is the sodium flux in the doubly perfused bullfrog kidney, which could easily be calculated from the data given by Swanson[28]. All our attempts to express Swanson's results on a comparable basis with the other figures has been in vain. We cannot find anybody who knows how many glomeruli there are per kidney in the bullfrog. Does anyone know this?

DR. OLIVER: I am sure no one does and certainly when you get into the human, no one has more than a vague guess, even as to the size or diameter or any other physical quality of the proximal convolution.

DR. GOODMAN: I would like to ask a question. Did previous workers add a different amount of mercury, for instance just a single drop put in for a collection experiment instead of a perfusion experiment, to explain why you do not find any net water or sodium reabsorption?

Or have any previous workers found reabsorption if they perfused the tubules instead of simply collecting?

DR. SOLOMON: Dr. Bott can speak to that better than I. Walker and Hudson obtained water reabsorption in collection experiments. With collections you don't need mercury. My recollection of Walker and Hudson's paper is that they rarely used a block of any kind distal to the collecting pipette, when they made collections. They simply inserted the pipette and collected fluid, so they very well might have been free of mercury. We used oil in all of our collections. We tried very hard to develop the oil technique to enable us to carry out perfusions with it. This would lay the ghost, finally.

On the whole, I feel relatively reassured from the battery of indirect evidence that has presented that mercury does not account for the lack of water reabsorption. Indeed, I would be surprised if it were mercury. However, the evidence comes only from this set of experiments, never quite enough and all of it really indirect.

DR. BOTT: I would have to review that. At the time most of those experiments were done, we did not have an ultra micro-inulin method suitable for use on tubule fluid, so that we did not have that as a measure of water reabsorption. The experiments in which glucose after phlorhizin was used[17] might give you some information on that but I am sure you have gone over this.

DR. METCOFF: Do you get any leakage around these pipettes?

DR. SOLOMON: The other gentlemen can answer this better than I.

DR. SHIPP: This is indeed a problem. There was no evident leakage around the pipettes in the reported experiments. Direct evidence that leakage occurs comes from the fact that fluid which collects on the surface around the pipettes contains significant amounts of radioactivity. The detection of fluid extravasation is readily apparent and if it occurs the experiment is stopped or discarded. We have considered only 26 out of 101 experiments to be technically satisfactory.

May I raise the question as to what effect the composition of the perfusing solution may have on sodium and water movement? In discussing this with Dr. Bott earlier this year she referred us to some studies on glucose reabsorption in the Necturus by Wood[43]. When the proximal tubule was perfused with a synthetic solution little or no glucose reabsorption occurred. Reabsorption was observed when pooled glomerular filtrate from the Necturus was used as the perfusate. The perfusing solution used in the reported studies has approximately the same sodium and potassium concentrations as the serum of the Necturus; chloride is present in higher, and bicarbonate in lower, concentrations. Calcium and phosphate are added but magnesium, glucose (except in those which contain C^{14} glucose) or amino acids are absent. May the inability to observe net water movement be referrable to the omission of a required factor or to the inclusion of an ionic species (or ionic concentration) which is inhibitory?

DR. BOTT: Do you have an oil layer on the surface of the kidney when you do this?

DR. SHIPP: In most of the experiments this was not done.

DR. BOTT: It prevents a certain amount of surface drying, imperceptible if the oil is not there.

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[43] Wood, E., Amer. J. of Physiol., 133:497, 1941.

DR. SHIPP: Oil is used in the current experiments.

DR. BOTT: Did you ever try the glomerular filtrate?

DR. SHIPP: We have not. Technically it is extremely difficult to collect and perfuse with the glomerular filtrate. This should be done, however. Another approach might be to use a plasma ultrafiltrate as the perfusing solution.

DR. FOX: Do I understand correctly that plasma ultrafiltrate is not as good as glomerular fluid?

DR. BOTT: That was not the remark that I made. Wood[44] tried all sorts of artificial perfusion fluids. I am not sure whether they tried macro ultrafiltrate or not, but I do know that artificial perfusion fluids did not work well whereas when collected glomerular filtrate was used the rate of glucose reabsorption was more independent of blood glucose level. They had to collect a lot of glomerular filtrate to perfuse. I know that is what you are thinking about. They used to set up Necturus with a collection going and would let it go all night and would sometimes be successful in keeping it going.

DR. FOX: How complete were the synthetic fluids they tried?

DR. BOTT: There is very little information on that because it was published, so far as I know, only in the Proceedings of the American Physiological Society. I believe there was a table in the abstract. However, I am not sure if anybody except Dr. Wood could give you the complete information on that.

DR. SOLOMON: In the case of the bullfrog kidney, the paper of Swanson[28] points out that glycine is a good substance to add to the perfusing fluid. I wonder if you have had experience with the addition of glycine.

DR. BOTT: That was in the old paper by Barkan, Broemser and Hahn[45] for the perfusion fluid. I used to omit it in my perfusion because I was studying proteins with a non-specific method, but it was used in most of the earlier perfusions in the Richards' laboratory.

DR. SHIPP: Almost all of the anion in the perfusing solution which we have used is chloride.

CHAIRMAN COOKE: Do you have a very low pH in the tubule? I take it that you do.

DR. SHIPP: This is probably correct but we did not do pH measurements. I would like to pursue a concept introduced by Dr. Metcoff this morning. Is the transtubular movement of protein unidirectional from tubule to blood? Or is there bidirectional transfer with the reabsorptive flux dominating? This is pertinent to the interpretation

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[44] Wood, E. H., Proc. Am. Physiol. Soc. 309:1941.
[45] Barkan, G., Broemser, P. and Hahn, A., Z. Biol. 74:1, 1921.

of protein concentration, as determined immunochemically, noted in tubular urine or collected fluid during perfusions. In earlier studies, Dr. Richards and Dr. Bott suggested that the presence of protein reflected damage to the tubule cells. Is the presence or absence of protein a good parameter of tubular integrity?

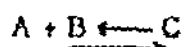
DR. METCOFF: There is another comment I would like to make relative to this work. That is the rather startling fact that sodium is presumably secreted into the tubule as "influx", if I understand correctly. People have been wondering about this for a long time. Would not a simple experiment be to inject radioactive sodium[24] into the efferent arteriole and simply measure it in the urine and see whether sodium influx really does occur in a larger animal?

CHAIRMAN COOKE: Aren't we dealing here with a problem that may not be a secretion? I thought one of the possibilities that always came up, at least according to Ussing's description, was that of exchange diffusion. There was simply swapping of labelled for unlabelled ions.

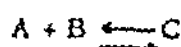
DR. METCOFF: Against a gradient?

CHAIRMAN COOKE: There is no work done in exchange diffusion at all. To have one particle swap with another does not require any effort whatsoever. We may have radioactivity moving against a gradient but that means nothing in terms of the sodium movement. There is no net movement and yet we have a great deal of shift across this membrane. Ussing makes a very strong point that one should not get confused and call it net transport necessarily when we see shift of radioactivity. It may simply be this phenomenon of exchange diffusion. I don't know how you would get around that here.

DR. SOLOMON: Most chemical reactions are reversible, as represented below:



Usually the equilibrium is shifted in one direction or the other, depending on the way the activated complex breaks down. If you will, you may split this reaction into 2 parts. You can call this part exchange diffusion,



where the equivalence of the arrows denotes that part of the reaction which may be considered to be reversible. On the other hand, the part of the reaction that might be represented by



is the excess of the forward over the back reaction. This part is commonly called active transport, if the forward reaction is up an electrochemical concentration gradient. It seems to me that the dichotomy involved in calling these 2 parts of the same reaction by different names introduces a very artificial way of looking at what must be, in the end, a series of chemical reactions. I would not say for a minute that in our measurements 1210 $\mu\mu$mol. of sodium have trotted the whole way across the membrane

and 1210 have trotted back. *In chemical terms what we have measured is an equilibrium* constant.

CHAIRMAN COOKE: This in no way suggests net secretion. That was Dr. Metcoff's point.

DR. SOLOMON: We don't have anything on net secretion.

CHAIRMAN COOKE: You have no evidence for secretion as we understand it, which means the net movement of material?

DR. SOLOMON: In terms of the net movement of material our *evidence is, I* think, inconclusive.

DR. SHIPP: We have two preliminary experiments which are pertinent to this part of the discussion. And, again, we apologize for the small number of experiments. In these experiments radio sodium (Na^{24}) was injected intravenously after the tubule had been blocked in the manner shown by Dr. Solomon. The tubule was then perfused with a solution containing no radioisotopes. Although not quantitated, the collected fluid showed significant amounts of radioactivity indicating movement of sodium from blood to lumen.

DR. SOLOMON: Certainly sodium ions move from one side to the other side, but the question as to how much work is done seems to me to remain completely open. For example, it seems to me quite important to make potential measurements, if we can ever learn how to do them under our conditions of perfusion, to see if a potential difference is present. That is the first step in answering this question.

DR. FOX: Would not some of the experiments of Chinard[46] be relevant here? *He used water containing* various isotopes of hydrogen and also isotopes of chloride, and showed the rapidity of exchange in both directions.

DR. SOLOMON: *The* advantage in using *2 isotopes is merely that you then avoid* the rather messy process of obtaining experimental results from the difference between 2 large quantities. It does not give you any information in a 2 compartment system. The Na^{23} that comes back into the tubule is just as marked as far as we are concerned, as it would be if it were Na^{22}, which is the other radioactive isotope of sodium.

DR. FOX: But you demonstrate it quite obviously.

DR. SOLOMON: You don't demonstrate anything more. You might make more measurements that are more significant.

DR. FOX: You see the movement in both directions.

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[46] Chinard, P. J. and Enns, T. Relative Rates of Passage of Deuterium and Tritium Oxides across Capillary Walls. Amer. J. Physiol. 178:203, 1954.

CHAIRMAN COOKE: It does not prove it any more than this work. You have shown without any question that there are sodium particles that are on one side that have gotten over on the other side of the tubule. That has nothing to do as far as I am concerned with demonstrating secretion, and four isotopes would not show it.

DR. SOLOMON: That is right. What we have measured is the flux in 2 directions. Assuming that my sets of equations describe the system adequately, then we have measured the flux in both directions across the tubule. Any conclusions beyond that are an unwarranted extrapolation from our data.

CHAIRMAN COOKE: If you could show a consistent influx, under certain circumstances, such as a mercurial diuresis, which was significantly larger than your efflux, then what Dr. Metcoff suggests is a possibility?

DR. SOLOMON: Yes, that is certainly true. There are a great many things we would like to do. Most of all, we would like to find a poison which would really affect the sodium efflux under our conditions. We are now busily engaged in trying to find out how much DNP is required to affect the urine of the Necturus. Then we hope to subject him to a large dose of DNP to see whether this has any effect upon sodium efflux from the tubule.

DR. BOTT: I think you answered one of the questions I had in mind. Since the efflux is greater than the influx in your normal animals you do consider that evidence of reabsorption?

DR. SOLOMON: That efflux is greater than the influx only by the grace of God, if you will. There were 7 experiments of which 3 were in one direction and 4 in the other direction. It just happens that the average falls the way we like it to fall. There is no demonstrable difference between those two numbers. They each bear large standard deviations and the fact that the one we would like to have larger than the other is indeed larger is merely a happy circumstance.

DR. BOTT: In all of these experiments the inulin was not concentrated?

DR. SOLOMON: No. In some of the experiments the inulin was concentrated. I think we obtained ratios as high as perhaps, 1.08 to 1.10. But for every positive value, there is another negative one. So on the average, we get a value of 1.

DR. FOX: What was the composition of the perfusion solution used.

DR. SOLOMON: The composition of the perfusion solution is given in Table XI.

TABLE 11

Concentration of Perfusate

Compound	mM/l
NaCl	92.0
KCl	3.0
$CaCl_2$	1.19
$NaHCO_3$	2.6
$NaH_2PO_4 \cdot H_2O$	0.94
Inulin	4 grams/l

Notes:

$Na^{24}Cl$ was added in addition to the NaCl concentration given in the table.

C^{14} glucose was added to a final concentration of 3.33 mM/liter in experiments in which glucose reabsorption was measured.

III. INVESTIGATIONS IN PROGRESS

CHAIRMAN BARNETT: For almost the first time since we have started these seminars we are going to carry out our initial intention to have a real unstructured, informal type of discussion concerning what our participants are doing research-wise, what they plan to do and what they are interested in at the present time. A number of people have incomplete data which are not completely analyzed. One of our purposes is to discuss such observations with one another while we are still collating and evaluating them.

Dr. Walter Heymann from Cleveland will start the discussion.

A. Dr. Walter Heymann

1. Antigen-antibody and properdin studies.

DR. WALTER HEYMANN: Let me first tell you about the immunological work that we have been doing during the last year. Unfortunately, it will be easy to report on this work in that, at least thus far, all our results have been negative.

The value of reporting these studies may be only that it might save someone else the work of doing it. These studies were stimulated by Dr. Najjar's[1] presentation in Buck Hill Falls, two years ago. He proposed a very attractive concept of antibody-antigen junction becoming antigenic again, forming a new antigenic complex. Another antibody joins that complex, becoming another antigenic complex, and so forth and so on. Inasmuch as no one thus far has succeeded in producing experimental renal disease by sensitizing animals with kidney tissue of the same species, we tried to simulate the possibility of such a complex mechanism as Najjar had proposed. In our laboratory, S. Harwood injected rats intraperitoneally three times weekly for a two-week period with blood free rat kidney emulsions. He then obtained the kidneys of this group of rats and injected them into another group of rats. The kidneys of this group, Number 2, were then injected in another group, and so forth and so on. By now he has the tenth passage in this study, and nothing has happened. Thus, we are ready to discontinue it.

DR. LANGE In the original rat, no antibody was stimulated at any time?

DR. HEYMANN: We did not go into any antibody studies. We only followed the urines of these animals and had histological studies of their kidneys done.

DR. LANGE Kidney emulsion of one generation rat given to the next?

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[1] Najjar, V. A., and Fisher, J. Mechanism of Antibody-Antigen Reaction, Science, 122 1272, 1955.

DR. HEYMANN: That is right.

DR. MITCHELL RUBIN: Did you ever take diseased kidneys and try to pass them?

DR. HEYMANN: We have done that, too. Nothing happens.

Another study I would like to mention derived from the observation that nephrotic children are highly susceptible to recurrent infections whereas this is not so in nephrotic rats. It is known that rats have a higher plasma properdin concentration than the human. It thus seemed of interest to study properdin concentrations in the plasma of nephrotic children and of nephrotic rats. Dr. Heinz, who works with Dr. Pillemer who in turn discovered properdin and developed the methods to determine its concentration, has studied this problem.

He found the plasma properdin concentration to be normal in nephrotic rats as well as in nephrotic children. The increased susceptibility to infections noted in nephrotic children thus is not due to properdin depletion.

DR. RAPOPORT: When you injected your rats in successive generations, how did you do this?

DR. HEYMANN: We perfused rat kidneys until they were blood-free, prepared an emulsion from the kidneys and injected it into other rats intraperitoneally. We have tried it intravenously, also. We have injected the emulsion intraperitoneally three times a week for two to three weeks.

DR. GOODMAN: You were not using Freund's adjuvant?

DR. HEYMANN: Without Freund's adjuvant. Then we killed that group of rats, obtained their kidneys and did the same thing in another group of rats. We hoped that if the first generation failed to form an antibody which is not pathogenic in its function with the antigen, maybe another generation would, but it didn't work. Is there any question on the properdin? I think the negative result is worth while knowing.

CHAIRMAN BARNETT: Is the concentration of gamma globulin reduced in rats as in the human disease.

DR. HEYMANN: I don't know. I couldn't say.

DR. METCOFF: Gamma globulin levels in rats are variable, depending upon the age and strain that you use. I remember years ago, when doing electrophoretic patterns on rats, that gamma globulin patterns were not altered with antibody production following a typhoid antigen -- even in protein-deficient rats.

DR. EDER: A partial answer to your question is that the same difference occurs between children and adults. I don't think there are any consistent differences between the gamma globulin levels in nephrotic children and adults.

CHAIRMAN BARNETT: This is rather interesting to me! Howard Eder and I have worked together on a paper and I was unaware of the fact that this great problem of susceptibility to infection, at least in edematous children, is distinctly different in adults. According to Dr. Eder's experience, the adult with nephrosis shows almost no increased susceptibility to infection. Has this been your experience also, Dr. Lange?

DR. LANGE: I have not been impressed by the susceptibility to infections in adults. I agree with Drs. Barnett and Eder.

DR. FOX: Apropos of properdin, Dr. Dubos had an interesting view. He felt that everything that Pillemer said was correct but he felt the cause and effect relationship had not been fully established. One of the changes which occurred with infection is a marked drop in the properdin level. It comes back in three or four days. He felt this was a concomitant of infection, like so many other changes that occur during infection, but it was not the factor which augmented susceptibility or prevented infection. Therefore this could be perfectly normal in an individual with marked susceptibility to infection and would not upset any concepts.

DR. HEYMANN: Which is the case, let us say, in the nephrotic individual.

DR. LANGE: I don't know whether this is the place to make a remark. We have tried to repeat Reed's experiments in relation to experimental nephritis in which he produced experimental nephritis in rabbits by injecting streptococci from individuals with nephritis. We have been unable to reproduce his results either with the strains that Dr. Ramelkamp sent us, or with Reed's strains, or the strains we have collected from the throats of individuals with nephritis. All of them, all B_{12} group streptococci, were completely negative as far as the production of experimental nephritis in rabbits was concerned. We have not produced nephritis in a single animal although we tripled and quadrupled the dose suggested.

DR. HEYMANN. In Copenhagen, a German group reported that a streptococcal strain that they obtained from Dr. Ramelkamp had produced glomerulonephritis in rabbits. I don't believe, however, that their results were convincing in that the renal lesions obtained were often of the kind associated with Schwartzman reactions.

2. Nephrotic renal disease in dogs

I now would like to give you a brief review of the experience we gained in producing nephrotoxic renal disease in dogs. We started this originally because we wanted the dog for studies of the hyperlipemia problem. We hoped to be able to obtain more blood and to obtain blood from the renal vein which could not be done with a small animal like the rat. If one uses anti-dog kidney serum obtained from rabbits, the resulting renal lesion in the dog differs from the disease obtained with similar sera injected into rats in several respects. First, as far as the clinical course is concerned there is an impressively higher incidence of progression of the renal disease to renal failure. From 20 dogs, three had a mild disease which subsided spontaneously within a few

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weeks, five had a moderately severe disease which lasted for four to six months and subsided spontaneously thereafter. 12 of the 20 animals, however, died in renal failure which is a high percentage compared with what we noted in rats. The symptomatology of the disease in dogs is also somewhat different. It is a nephrotic syndrome. They had marked proteinuria, no hematuria, hypoproteinemia and some hyperlipemia. Ascites was noted four times but edema of the extremities was never observed.

In the beginning we used puppies, later on we used young dogs five to six months of age. Hypoproteinemia was usually not as marked as in rats but values as low as 2.8 gm. per cent were observed. Hyperlipemia was always present but was of a moderate degree, not as marked as we had seen it in rats. It seemed possible that nephrotoxic serum obtained from another species would induce a more marked hyperlipemia. We thus injected dogs with nephrotoxic serum obtained from a goat and a horse. Five dogs injected with goat anti-dog kidney serum and six animals injected with horse anti-dog kidney serum were studied. The hyperlipemia obtained in these animals was not more pronounced than in the previous group. The worst values obtained were 600 mg. per cent for cholesterol and 2500 mg. per cent for total lipids. The corresponding control values for healthy dogs are 200 and 800 mg. per cent respectively. The higher incidence of the progression of the glomerular disease to renal failure noted in dogs seemed of interest. We knew from our studies in rats that if they are placed on a high protein intake, the incidence of resulting uremia may be increased at will. It thus seemed possible that the higher protein intake of dogs could account for the difference. Dogs kept on a grain diet developed progressive renal failure, however, as frequently as animals kept on a high protein intake. We feel justified, therefore, to assume that it is not the high protein intake which makes the disease more malignant in dogs.

DR. RAPOPORT: Did you get liver damage?

DR. HEYMANN: I do not know. Grossly we did not see any liver changes. The histological studies have not been obtained as yet.

DR. LANGE: Does the disease start immediately?

DR. HEYMANN: Immediately.

DR. LANGE: Drs. Fouts, Corcoran and Page have done work with dogs with sera from hens immunized against dog kidney and they have obtained a delayed disease. I think they used hens; anyway, it was an avian serum.

DR. HEYMANN: We have noted the same in rats injected with nephrotoxic serum obtained from ducks. A latent period was noted in these animals also.

DR. EDER: What was the salt content of the diet? Have you tried increasing the salt content of the diets?

DR. HEYMANN: I have not tried that.

DR. EDER: Dogs are very efficient in handling salt. More so than humans.

DR. RUBIN: How low did the serum albumin get? Did you get low albumin levels?

DR. HEYMANN. We got total serum protein values only. They went to 2.5, 2.8, 2.9 grams per cent, normal values being 5.5 grams per cent.

DR. BARNETT: The progression of the disease is associated with progressive loss of kidney function?

DR. HEYMANN: Yes, azotemia, creatinine retention, hypertension and frequently death in convulsions.

DR. FOX: Hypertension?

DR. HEYMANN: Yes, hypertension was noted occasionally.

DR. BARNETT: This information that you can produce this experimental model readily in dogs would be very useful to those whose interest is directed not so much toward the lipemia, but toward other aspects such as renal function and the problems of chronic renal insufficiency.

DR. HEYMANN: I believe that an interesting aspect of this work will be the implication that it has in reference to the classification of the nephrotic renal diseases. With the same etiological mechanism one can more or less induce whatever disease one wants to have, varying the diets or varying the animal species.

DR. LANGE: Walter, one statement that you just made in relation to Dr. Barnett's remarks in your last sentence I cannot accept, that is working "with the same etiological mechanism". This is not the same etiological mechanism. There is no auto-antibody involved in this type of disease. This is just kidney damage happening at the moment of injection which is followed by healing, scarring and its physiological sequelae.

DR. HEYMANN: I compared the rat disease to the disease obtained in dogs. Whatever happens in nephrotic children we do not know anyway.

DR. LAUSON: Has anyone yet used the Salk vaccine to try to produce the nephrotoxic disease in the monkey?

DR. HEYMANN: The Salk vaccine contains minimal amounts of kidney proteins. They do not seem to be sufficiently concentrated to be used as antigenic material.

DR. LAUSON: Maybe you can get some of the starting material.

DR. COOKE: That material has been injected. Prolonged injections into monkeys were made with no evidence of disease. That was one of the trials before they could let vaccine go out.

CHAIRMAN BARNETT. In the vaccine as it is prepared, there is an extremely small amount of kidney antigen.

DR. FOX: To follow up your point, it would seem to be very worth while with the availability of monkey kidneys to produce antiserum and use the monkey as the experimental animal instead of some of these animals so far from the subject of our conference.

DR. LAUSON: Sooner or later you ought to do this, don't you think so, Walter?

DR. HEYMANN: Monkeys are so disagreeable to work with; the collection of urine alone is a chore.

DR. FOX: Put them in a cage with a sloping floor and you get 100 per cent of the urine excreted.

DR. LANGE: You take blood every day on the monkey?

DR. FOX: In the monkey, femoral vein punctures are relatively simple.

DR. HEYMANN: I once started to work with monkeys and one of them got mad and you know what they do when they get mad at you. Since then I do not come close any more.

DR. FOX: There is one indispensable weapon. Take a tennis racket, cut the strings out, tie a bag on it and you can catch them readily. It is really not a problem. It is thought to be a problem until you start to do it.

DR. METCOFF: That is the best use for my tennis racket that I have heard of to date.

3. Aminonucleoside nephrosis

DR. HEYMANN: Maybe we can now discuss the aminonucleoside disease. Two years ago in Cleveland, Jack Metcoff and his co-workers reported that a derivative of puromycin, an amino-nucleoside, if injected in rats produces a nephrotic renal disease[2]. We repeated these experiments and confirmed all the findings that Metcoff and his co-workers have described. One interesting point is the latent period that is observed before the disease starts. If rats are injected every day subcutaneously with this agent, the disease starts after 9 to 13 days. We were interested in the latent period, in that it is unusual for a nephrotoxic chemical agent to produce such a long latent period. Bichloride of mercury, potassium bichromate or uranium nitrate produce an immediate renal lesion. We, therefore, first increased and decreased the latent period according to the dose used, to a certain extent, but never produced the disease immediately. Not even if one injected the agent intravenously.

Hemoglobinuria was noted immediately after the intravenous injection. This, however, subsided after a few hours. Thereafter the urine is protein-free for three or four days until the disease starts. The regular observation of a latent period suggested

[2] Metcoff, J., Craig, J., Frenk, S., and Antonowicz, I. Proc. 6th Annual Conf. on The Nephrotic Syndrome, 1955. Also cf. Proc. Soc. Exper. Biol. and Med. 89:424, 1955.

to us the possibility of an antibody-antigen reaction, the aminonucleoside rendering the kidney protein antigenic for some reason. Using the hemagglutination method described by Rothenberg and myself, we have failed to show the presence of renal antibodies in the sera obtained from aminonucleoside injected rats.

CHAIRMAN BARNETT: Complement has not been measured?

DR. HEYMANN: Yes, but I do not have the results as yet.

DR. KRETCHMER: Has anyone done any enzyme measurements in the latent period?

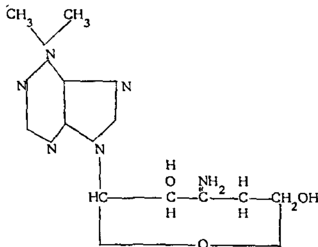
DR. METCOFF: We too thought that this form of nephrotoxic disease had an antigen-antibody phenomena. This is an area which I know so little about that I collaborated with Dr. Joe Ferrebee [3] who was kind enough to set up some studies to determine the possible antigenicity of the substance, to find out if any possible hapten interrelationship existed. He found none.

We also collaborated on some studies using C^{14} tagged acetate in relation to lipid metabolism, two years ago. These were completed but we were not entirely sure of the interpretation of the results. Dr. Ferrebee thought that the lipemia was due to newly synthesized cholesterol and fatty acids.

DR. HEYMANN: Tridione is another chemical agent that we know may produce the disease in children. The chemical formulas of these two compounds have, however, nothing in common. I have discussed this with Dr. Hans Hirschman, a very excellent chemist. The aminonucleoside is 6-Di-methyl aminopurine-3 Amino-D-ribose.

DR. FOX: Would you write out that formula, Jack?

DR. METCOFF:



6-Dimethylaminopurine 3 amino d-ribose

[3] Ferrebee, J., Clute, O., and Metcoff, J. Unpublished observations, October 1955.

DR. KRETCHMER: The interesting thing about the aminonucleoside is to consider whether it is a competitive inhibitor for protein synthesis.

DR. HEYMANN: That is a piece of work that Dr. Wilson is involved with. We are trying something of this sort. We are treating rats with adenosine and with ribose to see if it will prevent the disease.

DR. LAUSON: Have you tried the ten generation system on these kidneys to see whether they themselves might be somewhat antigenic when injected directly into another rat?

DR. HEYMANN: We have not done that. We have saved kidneys of rats treated with aminonucleoside, but we have not injected them as yet.

DR. GOODMAN: Did I understand that a single injection intravenously without further injection does have a latent period?

DR. HEYMANN: Yes.

DR. GOODMAN: When given subcutaneously, you give it daily. Can you give a single injection?

DR. HEYMANN: You mean subcutaneously? I don't know. I would guess if you give enough you probably could. That was six times the dose that we used daily with subcutaneous injections.

DR. RAPOPORT: What is the general natural history of this disease?

DR. HEYMANN: The natural history of the disease is that rats may develop a disease which may be so severe that they die within a few days after onset. If they survive, the disease subsides in 3 to 4 weeks and they are and stay well without recurrences. If, however, rats that recover were reinjected with the agent, a chronic disease of long duration could be produced.

DR. RUBIN: *What is the picture?*

DR. HEYMANN: Massive proteinuria without hematuria, marked hyperlipemia, hypoproteinemia and anasarca.

DR. LANGE: When you reinject does the disease appear immediately or has it again the same delay?

DR. HEYMANN: There is a shorter latent period. Instead of 11 to 13, it is maybe 5 to 7 days and then the rat keeps on with the chronic disease. We have some animals who up to date have had the renal lesion for four to five months with severe proteinuria, hyperlipemia, etc.

DR. McCRORY: Do they have liver damage?

DR. METCOFF: No significant liver damage occurs, at least not in the early disease.

DR. RUBIN: The first evidence is what?

DR. HEYMANN: Proteinuria starts slowly.

DR. RUBIN: That is how you judge the end of the latency period, by the increased amount of albuminuria?

DR. HEYMANN: Yes, there is no increased protein excretion in the urine until the disease starts. It then develops rapidly, reaching a maximum proteinuria within a few days.

DR. KRETCHMER: Do you continue the dose throughout the period?

DR. HEYMANN: With subcutaneous injections, yes. With intravenous injection, only one injection was given.

DR. FOX: Is aminonucleoside hemolytic in vitro?

DR. HEYMANN: Yes, it proved to be hemolytic in vitro.

DR. MICHAEL ROTHENBERG: That's right.

DR. FOX: What concentration was that?

DR. ROTHENBERG: 0.001 molar.

DR. FOX. Green and Stoner[4] have written a book on the adenine nucleotides. They have studied these extensively. There is evidence that ATP, ADP, IDP, ITP may be extremely important in many conditions where they have not been explored and in view of the evidence of cellular damage here it seems like a most important lead.

4. Pathogenesis of nephrotic hyperlipemia

DR. HEYMANN: I do not want to take up the whole morning and maybe we can finish up with a review of the work that we have done concerning the pathogenesis of the nephrotic hyperlipemia. I regret to see that Dr. Rosenman is not here today. You may remember that Drs. Rosenman, Friedman and Byers[5] suggested that hypoalbuminemia was responsible for the development of the hyperlipemia and they also proposed the theory that the hyperlipemia would be explained on the basis of an inability of the liver to take up plasma lipids, that plasma lipids were trapped in the plasma of nephrotoxic serum nephrotic rats. The latter assumption was attractive to me because we had found that in the nephrotic animals the liver lipids are significantly decreased.

[4] Green, H. N. and Stoner, H. B. The Adenine Nucleotides. H. K. Lewis & Co. Ltd., London, 1950.

[5] Rosenman, R. H., Friedman, M., and Byers, S. O. The Causal role of Plasma Albumin Deficiency in Experimental Nephrotic Hyperlipemia and Hypercholesteremia, J. Clin. Invest. 35:522, 1956.

DR. EDER: Drs. Gidez and James at Brookhaven found that the liver content of cholesterol was increased in aminonucleoside nephrosis in contrast to anti-rat-kidney nephrosis.

DR. METCOFF: Ferrebee found no difference between the heterologous serum nephroses and the aminonucleoside disease as far as liver C^{14} labelled chlosterol and fatty acids were concerned[3].

DR. HEYMANN: In order to test the hypothesis of "trapped" plasma lipids, plasma lipids that are not taken up by the liver tissue of nephrotic rats, Drs. Matthews, Bergman and myself injected 5 control rats and 5 nephrotic rats with a C^{14} labeled trilaurin emulsion intravenously. We killed them after 1, 2, 4, 6, 8 and 24 hours and the clearance of the trilaurin from the blood yielded identical curves for both groups. We had found that previously and we confirmed it again this time. This time, however, we also investigated the up-take of the tagged fatty acid by the liver, by the kidney and the remaining carcass. I do not have the carcass values as yet, but the nephrotic livers took up trilaurin as quickly and intensively as livers obtained from control animals. For the period of time tested and for that particular fatty acid used we have found no evidence that indicates an impaired ability of the nephrotic liver to take up this short chained fatty acid.

We have followed up the problem of the hypoalbuminemia as the responsible factor eliciting the nephrotic hyperlipemia. You may remember that Rosenman, Friedman and Byers[5] implanted the ureter of rats on one side into the lower vena cava and ligated the ureter of the other side, so that no protein-containing urine was lost. Under these conditions the hyperlipemia was markedly reduced or did not occur after the injection of nephrotoxic sera. They furthermore found that the intravenous infusion of nephrotic rats with albumin decreased the hyperlipemia. They concluded that the hyperlipemia results from the decreased concentration of plasma albumin.

We have, thus far, approached this problem in three different ways. First, we determined the serum albumin concentration and the total lipid and cholesterol concentration in the plasma of nephrotic rats with a well-established disease. We noted no correlation between the degree of hyperlipemia and hypoalbuminemia. This does not exclude the possible validity of the hypoalbuminemia hypothesis, but our findings certainly do not support this hypothesis in any way.

In another study we injected rats with nephrotoxic sera and followed the development of the hyperlipemia. If the hypoalbuminemia is the cause of the hyperlipemia, one should expect the former to precede the latter. Thus far we have 5 rats that were killed 2, 4, 6, 8 and 24 hours after the injection of the nephrotoxic sera. In one animal the decrease in the serum albumin preceded the development of an increase in the lipids by an hour or two. In another rat there was hyperlipemia developing without any decrease of serum albumin. In 2 animals the hyperlipemia definitely preceded the development of hypoalbuminemia, and in one rat they developed simultaneously. We thus feel that both hypoalbuminemia and hyperlipemia are manifestations of the disease process, but it seems difficult to establish a causal relationship between the two.

DR. JAMES H. BAXTER: Have you found the lack of relationship in patients, too?

DR. HEYMANN: I have not studied that with serum albumin determinations.

DR. BAXTER: We have given albumin infusions to nephrotic patients with hypoalbuminemia and hypercholesterolemia and followed serum albumin and lipid levels. As the serum albumin rises as a result of the infusions, the levels of cholesterol and other lipids fall, though the quantitative relationships between serum albumin and serum lipids at various levels of albumin is not the same from one patient to another or from time to time in the same patient. These observations suggest that the hyperlipemia may be at least in part secondary to the hypoalbuminemia.

DR. HEYMANN: Did you observe the same with dextran?

DR. BAXTER: I did not.

DR. HEYMANN: If one increases the oncotic pressure with any agent, would it have any effect on the hyperlipemia? I would not deduce too much from the effect of albumin unless I would know that.

DR. METCOFF: Hyperoncotic dextran does not change the cholesterol levels beyond the factor of dilution, and this is a very temporary change.

DR. HEYMANN: If you get them to diurese with dextran?

DR. METCOFF: Then cholesterol levels change.

DR. BLAINEY: We have a fair amount of data suggesting a correlation, I could show you the slide later, between cholesterol and serum albumin during the spontaneous course of the nephrotic syndrome in adults. We have also observed the effect of albumin infusion.

DR. HEYMANN: As we pointed out before, that says little about a causal relationship.

DR. BLAINEY: Right.

DR. HEYMANN: Both are symptoms of the disease. If you improved them both it still would not prove that the one caused the other.

DR. LAUSON: I recall work that was done several years ago by Knutti et al. in which gum acacia was administered to dogs following plasmapheresis[6]. They found that total colloid osmotic pressure remained normal while the concentrations of albumin and acacia varied inversely under the various conditions of their experiments. If they had obtained data on plasma lipids, these data would be good to have for discussion.

[6] Knutti, R. E., Warrick, R. A. and Goetsch, J. B., Maintenance of blood colloid: passage of stored gum acacia from cells to circulation after plasmapheresis. J. Exper. Med. 92:77, 1950.

DR. HEYMANN: I doubt that they would clarify the issue because dogs do not produce this type of hyperlipemia readily.

DR. COOKE: There is an experiment of nature going on in children at the present time that I think all of us are seeing somewhat more frequently. That is idiopathic hypoproteinemia in which there is marked edema, hypoalbuminemia and hypoglobulinemia and in our experience frequently no or little hyperlipemia. I think one can certainly not relate hyperlipemia necessarily directly to hyperalbuminemia.

DR. HEYMANN: There is no hyperlipemia?

DR. COOKE: Right.

DR. EDER: Are the children well nourished who have this syndrome?

DR. COOKE: The usual history is that of excellent nutrition, yes.

DR. McCRORY: The degree of hypoalbuminemia in idiopathic hypoproteinemia is usually not as marked as that found in nephrosis where serum albumin is 0.2-0.3 gm.%. In the few patients we have seen the level of serum albumin was 1 or 1.5 gm.%. I should think this might be important.

CHAIRMAN BARNETT: In addition, in patients with nephrosis who have the degree of hypoalbuminemia that occurs in idiopathic hypoproteinemia you almost regularly see elevation of lipids.

DR. GOODMAN: All I can say bearing on this is that we have seen 2 patients studied by others who had idiopathic hypoalbuminemia. They had serum albumins of less than 100 mgm.% with only a mild elevation of serum cholesterol. It was nothing like the hyperlipemia seen in some nephrotics.

DR. HEYMANN: That is right.

DR. McCRORY: Are you claiming that there is no relationship between the occurence of lipemia and hypoalbuminemia in nephrosis because hypoalbuminemia is seen in some hypoproteinemic states without lipemia?

DR. HEYMANN: I doubt that a causal relationship has been established.

DR. RAPOPORT: I am sure other people have seen such patients and I am sure we have seen more than one. The one I am thinking about is one on whom Dr. McCrory did a lot of work while she was hypertensive and who later died. This child was not amenable to any sort of treatment other than trying to keep the blood pressure down and she was edematous all the time. Her serum proteins stayed low and then for no reason at all her cholesterol drifted down to normal and stayed normal without any change in anything else. She died with all the signs of nephrosis, but a normal cholesterol. It has always been my opinion that these are simultaneous phenomena but not causally related one to another.

DR. HEYMANN: That is right. We see that in the rat disease also.

DR. LITMAN: Would there be some importance in the fact that hypoproteinemic patients have excessive protein catabolism and this is not the situation in nephrotics?

DR. HEYMANN: The phase of increased protein catabolism, as found by Gitlin, Janeway and Farr[7], may be a point of important difference.

I want to close with another group of experiments. They refer to the studies of Rosenman, Friedman and Byers[5] where in nephrotic rats the ureters of one side were implanted in the lower vena cava while the contralateral ureter had been previously ligated. This prevented the development of the hyperlipemia. The important control to this experiment could not be obtained and that is the effect that normal non-protein containing urine may have on the nephrotic hyperlipemia. Urine is not an entirely innocuous fluid if injected intravenously. In this study we took 8 nephrotic rats that were injected with nephrotic rat urines. Another 8 rats were injected with normal rat urine intraperitoneally. They were injected twice daily with 4 cc. of urine which was well absorbed. Eight nephrotic animals were injected with saline, and at present we are injecting 8 rats with dextran solution. From the 8 rats injected with nephrotic urines, 8 had a marked decrease in total lipid concentration in their plasma. From the 8 nephrotic rats injected with control urines, 5 showed a decrease in plasma lipid concentration of equal magnitude. From 8 nephrotic animals injected with equal amounts of saline, only 2 showed an unquestioned effect on their hyperlipemia. The cholesterolemia responded with the same pattern. At present we control these results with dextran and also in 8 nephrotic animals that do not receive any injections but that are bled on the same schedules. From the results thus far available, it is possible that the infusion of nephrotic urine into the nephrotic animals of Rosenman, Friedman and Byers resulting from the ureter-vena cava anastomosis decreased the blood lipid concentration not only because of the introduction of albumin into their bloodstream, but because of other substances contained in the urine.

DR. KAPLAN: Would it not be wrong to assume that all the protein is albumin in the urine? You should use a control with albumin removed from the nephrotic urine.

DR. HEYMANN. I believe this is partly answered by using normal, protein-free urine which reduced nephrotic hyperlipemia 5 times out of 8 equally well.

DR. LAUSON: You take the control sample something like 6 or 8 days prior to injection of urine?

DR. HEYMANN: We bled the animals and started to inject them with urine four or five days later. We injected them for eight days. Eight cc. per day., i.e., 4 cc. two times daily. The urine was pooled and sterilized.

DR. LAUSON. I should think that a nephrotic rat would put out small volumes of more concentrated urine. Therefore 4 cc. of such urine would represent more hours of excretion than 4 cc. from a normal rat excreting a larger volume per hour.

[7] Gitlin, D., Janeway, C. A., and Farr, L. E., J. Clin. Invest. 35:44, 1956.

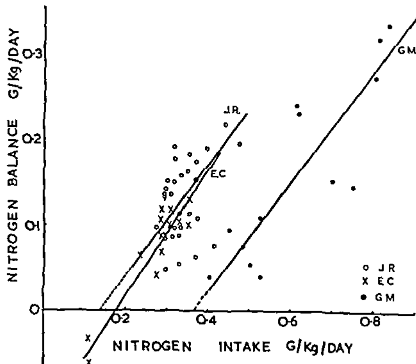


Fig. 24. Relation between nitrogen balance and intake in three patients. Individual points relate mean intake and balance for each 3-day period per kg. standard body weight. The regression lines are drawn for each case. (Reproduced from Blainey, J. D., Clin. Sci. 13 567, 1954.)

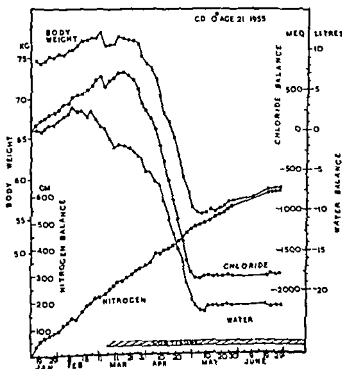


Fig. 25. Cumulative balance data in 20 year old male with nephrotic syndrome. Arbitrary zero points chosen at the beginning of the balance period, and scales chosen so that 1 kg. weight = 1 liter water = 104 meq. chloride = 30 g. nitrogen. (See text.)

DR. HEYMANN: I don't think so, because these urines were not obtained from edematous nephrotic rats. Their urinary output and volume is the same as in control animals.

DR. LAUSON: I see.

DR. FOX: Didn't Wesson inject large volumes of urine intravenously in dogs and found it was amazingly innocuous?

DR. COOKE: Wesson kept up the electrolyte content of the body at least by putting back what they excreted. I don't think they made any measurements on lipids.

DR. HEYMANN: Clinically the rats injected with urine did very well also.

CHAIRMAN BARNETT: Thank you very much. It is always a pleasure to me to hear the amount of work that is going on in Dr. Heymann's laboratory.

I wonder if I might now ask Dr. Blainey whom we are glad to have with us. if he would tell us some of the things he has been doing.

B. Dr. John Blainey

1. Metabolism of nitrogen in nephrotic patients

DR. BLAINEY: I should like to take the discussion back to the clinical aspects which is what we have been studying more than the fascinating experimental work that we have just heard about. In Birmingham, we have been interested in the profound muscle wasting and in the very great losses of protein that occur in the urine in many of our patients, both of which suggested severe loss of protein stores from the body.

We repeated the studies of Farr[8] on the effects of different levels of nitrogen intake upon the nitrogen balance. Figure 24 shows the data obtained in three cases with the nephrotic syndrome and severe proteinuria[9]. We fed these patients with different levels of nitrogen intake ranging from 0.2 g./kg./day to 0.8 g./kg./day (60 to 200 g. protein per day). Case I was a girl of 20, Case II an elderly man and Case III a girl of 14. The graph shows that we did not obtain the optimal effect observed by Farr from which he concluded that nitrogen intakes above 0.5 g./kg./day caused an actual reduction in the positive nitrogen balance. In our cases it was noticed, however, that after each successive increase of diet there was a temporary period of 3 to 9 days during which the balance of nitrogen retained in the body did decrease for a given intake. After this short period of equilibration, the balance again rose to the expected levels.

DR. GOODMAN: While they were edematous and had the nephrotic syndrome?

DR. BLAINEY: Yes. All three were edematous and although two had a diuresis during the period of study, it made no difference to the nitrogen balances.

[8] Farr, L. E., Amer. J. Med. Sci. 195:70, 1938.
[9] Blainey, J. D., Clin. Sci., 13:567, 1954.

DR. RUBIN: Was the caloric intake kept the same?

DR. BLAINEY: No. During the study the caloric intake was varied as a result of increasing both calories and nitrogen together and it is not therefore possible to differentiate the calory effect and the protein effect upon the nitrogen balance. Subsequent unpublished studies on other patients have shown that increase of calories alone increases the nitrogen utilization slightly. Thus in Figure 24 the slope indicates approximately 70 per cent utilization of fed protein; doubling the calory intake with a constant nitrogen has shown a rise to about 80 per cent.

Figure 25 shows the very large positive nitrogen balance observed in one of these patients. This young man of 21 gained nearly 600 g. nitrogen in the six month period of balance studies. These cumulative balances do, of course, include the possibility of large errors but we have tried to reduce these as far as possible by careful metabolic ward study. This slide raises many other points regarding the correlation between nitrogen, body weight, water and electrolytes but I would like to emphasize once again the degree of depletion of protein that many of these patients show when they are first seen.

DR. FOX: Did you have a K to N ratio?

DR. BLAINEY: No. This graph does not show K:N ratios. The patient was in positive potassium balance throughout, but for the first 10-15 three-day periods the potassium to nitrogen ratio is about two to three times the usually quoted figure of 2.7. The ratio, however, gradually declined to a more normal level towards the end of the period of study. We have found somewhat similar results in several other patients.

The data of these cumulative balances is plotted on scales so that the weight, nitrogen, chloride and water are comparable. Thus, nitrogen assumes the usual 30:1 ratio; the chloride scale is based upon a linear regression between water and chloride from which the slope indicates that 1 liter water (1 kg. weight) is equivalent to 104 m.eq. chloride. The cumulative water balance includes a mean daily allowance of 1.03 liters for insensible loss: this figure again is derived from the intercept of the same regression. I had not intended to discuss the electrolyte changes in detail as I am at the moment completing the analysis of data on 5 patients.

There is a close positive correlation between chloride, sodium and water and between these parameters and body weight.

The chloride balance and water balance run together except for a period at the onset of diuresis when more water is lost than would be expected from extracellular fluid analysis. The data for the later periods suggests loss of fluid of extracellular fluid composition.

DR. FOX: That is a very important point. There has been a great deal of discussion about the isotonicity of diuresis fluid. If the data are consistent one can only infer there is more water than salt.

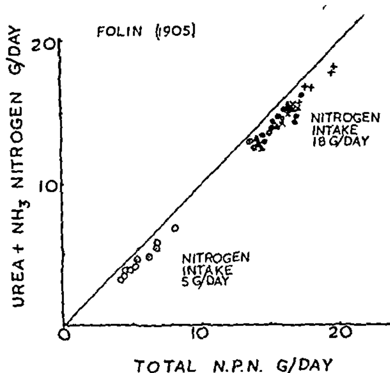


Fig 26. Relation between urea + ammonia nitrogen and urine N P N. data from normal subjects (after Folin.)

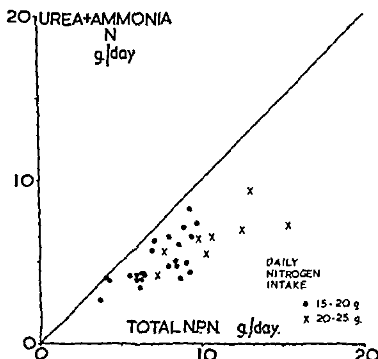


Fig 27. Nephrotic patient with amino-aciduria and excessive urinary nitrogen loss. (Reproduced from Blainey, J. D., Clin. Sci. 13.567, 1954.)

DR. BLAINEY: We were principally interested in where all the nitrogen goes to, since it is obvious that it does not go into the serum proteins. This is one of the reasons why the value of high protein feeding has been so often questioned. One further source of nitrogen loss in the urine must be mentioned since it has a bearing on the utilization of fed protein. You are familiar with the classical studies of Folin[10] who showed that the urea plus ammonia nitrogen excretion was a remarkably constant fraction of the urine nitrogen in the absence of proteinuria. The total daily excretion of nitrogen depends upon the nitrogen intake, and rises and falls in a predictable manner in normal subjects (Figure 26). With several patients we have observed large increases in non-protein, non-urea nitrogen excretion on raising the protein intake (Figure 27). Most of this increase can be accounted for by excessive amino-acid excretion, but on occasions there has been 1-2 g. unaccounted for nitrogen in the urine.

DR. FOX: Do you recall the per cent of the nitrogen which is ammonia? Is that a constant or changing value?

DR. BLAINEY: It is constant unless you alter acid-base balance in any way. Then as originally shown by Gamble[11] there is a reciprocal relationship between urea and ammonia nitrogen. We found considerable increase in the ammonia excretion in a patient with the nephrotic syndrome on giving calcium chloride, but the urea + ammonia remained constant.

To return to the fate of the nitrogen actually retained in the body. Figure 28 relates serum protein to proteinuria in four separate patients in whom we have also done nitrogen balances. It is clear that there is no appreciable increase in the serum albumin concentration until the proteinuria diminishes. There is, however, some increase in the proteinuria in cases (a) and (b) on increasing protein intake in hospital. The dotted line in the graph represents theoretical limits to normal serum albumin production[12], and it is seen that initially cases (a) and (c) fell below these lines, suggesting decreased albumin production. Case (c), an 8 year old boy with the nephrotic syndrome, was unusual in that on high protein diets he excreted more urea nitrogen than expected, i.e., there was only a small positive nitrogen balance. The albumin production remained low until he was treated with cortisone. There was no obvious explanation, such as intercurrent infection, which might have caused this unusual picture.

DR. BARNETT: Have you made similar observations on other children? This was the only child in the group?

DR. BLAINEY: This is the third child on whom we have made similar studies.

DR. RAPOPORT: What did you mean when you tried to relate this to intercurrent infection?

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[10] Folin, O., Amer. J. Physiol 13:45, 1905.
[11] Gamble, J. L., Chemical Anatomy, Physiology and Pathology of the Extracellular Fluid, Harvard, Mass., 1952.
[12] Squire, J. R., Amer. J. Clin. Nutrit. 4:509, 1956.

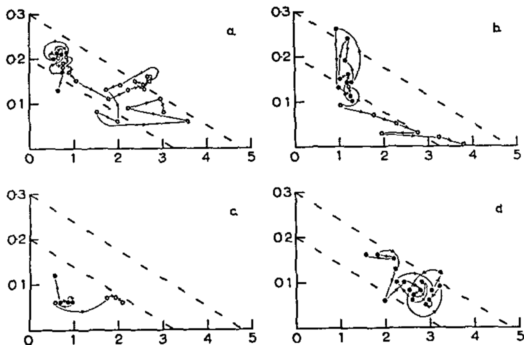


Fig. 28. Relation between serum albumin concentration and urinary albumin loss in four different patients showing successive determinations over many months.

Ordinate - Albumin loss in urine in g/kg. body weight/day.

Abscissa - Plasma albumin concentration, in g/100 ml. Solid dots - conservative therapy. Open circles - cortisone treatment. (Reproduced from Squire, J. R. et al., Brit. Med. Bull., in press.)

DR. BLAINEY: We were principally interested in where all the nitrogen goes to, since it is obvious that it does not go into the serum proteins. This is one of the reasons why the value of high protein feeding has been so often questioned. One further source of nitrogen loss in the urine must be mentioned since it has a bearing on the utilization of fed protein. You are familiar with the classical studies of Folin[10] who showed that the urea plus ammonia nitrogen excretion was a remarkably constant fraction of the urine nitrogen in the absence of proteinuria. The total daily excretion of nitrogen depends upon the nitrogen intake, and rises and falls in a predictable manner in normal subjects (Figure 26). With several patients we have observed large increases in non-protein, non-urea nitrogen excretion on raising the protein intake (Figure 27). Most of this increase can be accounted for by excessive amino-acid excretion, but on occasions there has been 1-2 g. unaccounted for nitrogen in the urine.

DR. FOX: Do you recall the per cent of the nitrogen which is ammonia? Is that a constant or changing value?

DR. BLAINEY: It is constant unless you alter acid-base balance in any way. Then as originally shown by Gamble[11] there is a reciprocal relationship between urea and ammonia nitrogen. We found considerable increase in the ammonia excretion in a patient with the nephrotic syndrome on giving calcium chloride, but the urea + ammonia remained constant.

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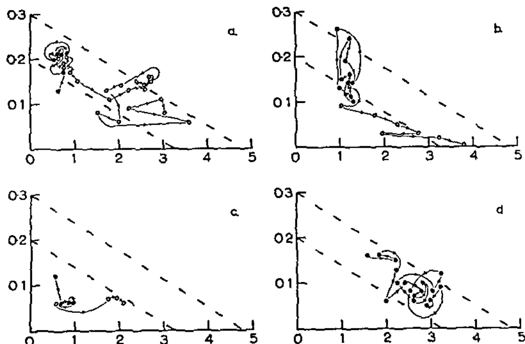


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DR. BLAINEY: There was no obvious evidence of infection. We have, however, found a definite leucocytosis in a number of patients with the nephrotic syndrome in the absence of other classical signs of infection.

DR. RAPOPORT: With or without cortisone?

DR. BLAINEY: I don't think the cortisone has anything to do with it. We see it in patients with cortisone and without cortisone.

DR. RAPOPORT: Just on very little evidence I have gotten around to changing antibiotics and giving large doses of whatever I change to when things start to happen almost imperceptibly. I just sent a child home who came in to have albumin infusions, who was getting cortisone, and although nothing happened to him he did not seem well and then one morning had a large painful area on his thigh with no rise in his temperature. He had a positive blood culture. He was getting cortisone. This is something everybody knows and it is getting increasingly difficult to find out when they are infected.

DR. FOX: Do you have any serum bicarbonate values if you change the protein level of the diet?

DR. BLAINEY: I have not found any substantial change. I think if you suddenly increase the protein intake, there is this curious time lag in the excretion of urea. It takes about four days before the urea excretion comes up along the curve. During those couple of days you might get a slight shift of bicarbonate. I don't think it is significant.

DR. FOX: The reason I ask, Stewart in Edinburgh made some interesting studies about 25 years ago on the effect of high protein diets in nephritis. Apparently he found a rather marked change in the acid-base balance and that the most deleterious effect of the high protein diet in nephritis was the increase in acid metabolites which had to be excreted as you increase the protein.

DR. BLAINEY: We did not find that. I don't think the fairly steady ammonia excretion would support that. These people are able to form ammonia. We gave ammonium chloride once and got the exptected response in ammonia excretion.

DR. METCOFF: I cannot really leave the nitrogen balance data go without some comment. Many studies have been done in the nephrotic syndrome and they are always rather perplexing. This is particularly true in regard to nitrogen balances. It is rare that one finds a negative nitrogen balance in the nephrotic syndrome, yet as you point out, muscle wasting is a characteristic feature. It is rather curious. It is also curious that if one analyzes the muscle of nephrotic animals, for example, the non-collagenous nitrogen concentration in them is normal. This one patient whose detailed balance you showed us should have gained about 20 kilograms of tissue, according to my quick calculation.

DR. BLAINEY: Yes.

DR. METCOFF: Twenty kilograms of tissue during a time when he lost 15 kilograms of weight, presumably of edema fluid. This also meant that his body potassium,

assuming a reasonably constant ratio between potassium and nitrogen, should have doubled. This is very curious, and I really wonder what protracted balance measurements mean in the first place; where the error is, in the second place, and whether it is fair to draw too many inferential conclusions from them, in the third place.

DR. BLAINEY: I think that the weight changes in this patient are rather greater (Figure 25) and the positive potassium balances are very large. The patients appear to be gaining a good deal more nitrogen than the usual nitrogen to weight ratio would indicate, and the slope of the nitrogen balance on the figure is therefore too steep. Although this type of data is difficult to explain, it has all been obtained in the Metabolic Ward where the protein intakes have been largely in the form of dried milk powders which have been repeatedly analyzed. Fecal collections have also been frequently checked.

DR. METCOFF: I have no doubt about the collections or measurements because I think all people who have done careful balances have come to the same conclusions. They are so similar that I doubt whether they are erroneous. I am not implying errors. I just don't understand why or where.

DR. BLAINEY: In these states of muscle depletion I think we have to reject altogether the analysis of normal muscle tissue. The muscles that you analyze, after all, in your muscle biopsy are the remaining normal muscles. We don't know what has gone into wasted muscles in which whole fibers may well have degenerated altogether.

DR. METCOFF: To settle that particular point, which we thought rather important, since there was little available data on the subject that we could find, we did total body non-collagenous nitrogens in carefully pair-fed nephrotic rats. We found that their total non-collagenous nitrogen[13] was significantly reduced. This was not surprising, although the non-collagenous nitrogen content in muscle was normal. That is just as you suggest, the remaining fibers at least had a normal nitrogen composition, but presumably large areas of muscle fiber had disappeared. However, it is difficult to understand, then, how large masses of muscle could disappear and yet with nitrogen intake maintained, how the nitrogen balance could remain positive. Do cells become supernitrogenated?

DR. BLAINEY: I think that one of the explanations may be that many of these patients are on poor protein intakes before they come to hospital. I imagine that your nephrotic rats are not kept on severely depleted protein intakes before you do these muscle analyses? It might be interesting to do that and to see whether one could vary the potassium:nitrogen ratio in muscle.

DR. EDER: Could you comment on the clinical effect of high protein diets? Prof. Max Rosenheim was in this country last spring and he stated that he believed that diuresis was induced in some patients when high protein diets were used.

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[13] Metcoff, J., Observations on the Renal Defense of Body Composition in Children. Amer. J. Clin. Nutrition, 4:543, 1956.

DR. BLAINEY: That is true. The explanation may be that many of these patients are producing albumin at less than normal rates before they are put on therapeutic diets. They are sick, edematous and have very poor appetites and their nitrogen intakes may be very low indeed. This may be one reason why we do not see negative nitrogen balances in hospital, as there we always give a higher protein intake.

DR. EDER: This is without rise in serum protein.

DR. BLAINEY: Increase in plasma volume and total circulating protein seems to occur and to result in diuresis, often without much change in the serum albumin concentration.

DR. RAPOPORT: May I add a few clinical observations? I won't swear to them. There are no quantitative data on these. In the days when nephrotics stayed nephrotics it impressed me they never needed a haircut and rarely had to cut their finger nails. They could go for months between haircuts and actually if you compared them to other children who wasted, such as babies who had miliary tuberculosis, or small children of that type, their hair was growing down over their face. In addition they had a brow which started to get narrower because hair grew down in front of the hair line. The children with severe wasting diseases had an increase in the catabolic phase. The nephrotic may have some of this but he also has what I guess, until somebody disproves it, some anabolic failure and that his protein wasting is probably due to a relatively normal amount of catabolism or, if anything, a slight increase. But it is exaggerated by failure of replacement.

DR. BLAINEY: That may be so. Certainly the hair growth we would confirm. We have seen that. I tried rather unsuccessfully to measure the growth rate of hair.

CHAIRMAN BARNETT: You are saying that the rate of synthesis of proteins is decreased?

DR. RAPOPORT: Yes. I purposely omitted stating serum proteins. All the evidence that seems to be presented from isotope studies and others is that this isn't retarded. But certainly here is a tissue you can see and if the child needed a haircut once a month, that period can extend to eight months without a haircut.

DR. FOX: We don't know what makes hair grow. There are lots of people, well nourished, with no hair. This is not even qualitative data, Milton.

DR. RAPOPORT: Qualitative, yes I think it is.

DR. METCOFF: I would like to come to Milton's defense. In children with the nephrotic syndrome retarded hair growth is a very characteristic sign and oftentimes one can determine the activity of the disease by simply asking the mother when the child had his last haircut. She will often say, "You know, it is a strange thing but about a month ago Johnny's hair started to grow again" and sure enough, Johnny's cholesterol is now down, the serum protein is up, and remission is evident.

DR. FOX: There is no question about the fact, the question is with interpretation. Hair growth may be a function of the endocrines, which has nothing to do with the cellular metabolism.

DR. METCOFF: In severe malnutrition, such as kwashiorkor, which usually occurs in children between the ages of 2 and 4, there is no cessation of hair growth.

DR. HEYMANN: May I add one intriguing observation which we made recently? Nephrotic children have an amazingly low resistance, or elasticity, in the cartilagenous structure of their ears. The cartilage feels flabby like the cartilage-free ear lobes do. In terms of time relationship it goes hand in hand with the growth of their hair. It takes two, three or four weeks of a good remission for the elasticity of their ears to return to normal. Have you noticed that?

DR. RAPOPORT: I have not noticed that.

DR. METCOFF: Perhaps this is due to increased water in the cartilage.

DR. HEYMANN: I do not believe so. If they had diuresed completely the ears are still flabby.

DR. METCOFF: That may be called the Heymann sign.

DR. HEYMANN: Abbott drew attention to it.

CHAIRMAN BARNETT: I think we should go on to some of the work that is being done in some of the other laboratories. I would like to ask Dr. Lange if he would tell us about some of the observations he has in progress.

C. Dr. Kurt Lange

1. Studies on capillary permeability models

DR. LANGE: There is a group of observations which started from a discussion at the breakfast table with Bob Cooke. We discussed the origin of edema in nephritis. We all agreed that the classical idea that this edema is due exclusively or predominantly to the lowering of plasma proteins is not tenable. I think this hardly needs any discussion in this group. We all have seen children diurese and diurese completely without any rise in plasma proteins and on the other hand, other nephrotics who became severely edematous without having initially a fall in plasma protein which decreased only later on.

CHAIRMAN BARNETT: If you are implying that the concentration of plasma proteins has nothing to do with edema --

DR. LANGE: No, I don't, Henry, I beg your pardon. I do not wish to say that it has no part in it at all, I only wish to say that it is not the only cause of the edema. Dr. Goodman has just told me that in a case of spontaneous hypoalbuminemia with 600 mg. % albumin in the blood which they had at the N.I.H. there was very little edema.

In one case of ours with the same disease and also a very low serum albumin level there was just a small amount of periorbital edema but no other edema.

This observation of edema which cannot be explained by the classical Starling theory, started us out many years ago for the search of a factor which could cause increased capillary permeability. Thus we looked for an antigen-antibody reaction playing a role in the alteration of capillary permeability as is seen so classically in serum sickness.

Capillary permeability requires a more precise definition. The term capillary permeability is generally used loosely and most observers understand it to be permeability to proteins. What we mean is permeability to water and from the experiments which we will show you, you will note that these experiments are mostly directed toward the study of capillary permeability to water and its relation to capillary permeability to proteins.

In Starling's classical theory of the mechanism of edema formation two factors are considered, namely, hydrostatic and osmotic pressure. We have learned in the meantime about the significance of sodium retention for edema formation.

We built a rather simple model (Figure 29) which consists of a water reservoir which is kept at a constant level. The water from the reservoir flows into a porous porcelain candle; such candles may be obtained commercially with different degrees of porosity. The porcelain candle is exchangeable in the setup and is the centerpiece of a chamber, the walls of which are formed by condom rubber, the outer chamber has a small outflow opening, corresponding to the lymphatics. Initially, no semi-permeable membrane was used in the porous candle. The actual pressure in the outer chamber is measured by a manometer and represents the counterpart of the pressure in the tissue space. When one exchanges the candle with a low porosity for one with a high porosity the pressure in the outer chamber limited by the condom rubber rises and the condom rubber bulges out, which represents a counterpart to edema formation. Please note that there is no change in hydrostatic pressure since the water reservoir wasn't raised. Only by a change of permeability to water, edema was produced.

In the next step we produced collodion membranes over the inner surface of the candle. The porosity of such collodion membrane can be varied at will, by shortening or lengthening the drying time of the membrane or by adding more or less ether to the collodion. Thus one can produce a membrane with any desired permeability. Such membranes can be made with a porosity which corresponds approximately to the porosity of skin capillaries (max. permeability mol. wt. 1,000) (type 1) and to a porosity which just permits albumin to leak (max. perm. porosity mol. wt. 60,000) (type 2). We are fully aware that molecular weight is not the only determining factor in permeability and that we have omitted for the sake of simplicity such important factors as electrical charge, molecular shape, etc. When the porcelain candle was covered with the collodion membrane of type 2 (max. perm. mol. wt. 60,000) which just permits albumin to go through, this candle permitted 26 times as much water to go through per minute than when a candle of type 1 (max. perm. mol. st. 1,000) was inserted; that would mean that a skin capillary can increase its permeability to water 26 times before albumin starts to leak through. Assuming that in this system (type 1) there was nor-

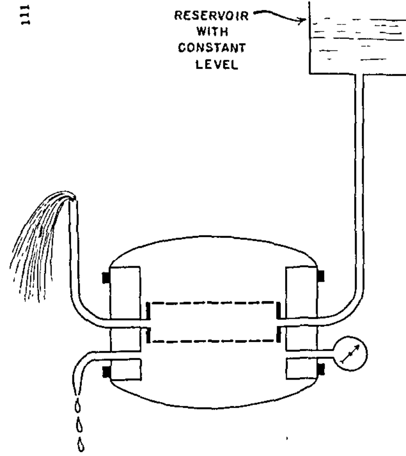


Fig. 29. Capillary *permeability* model using a porous porcelain candle.

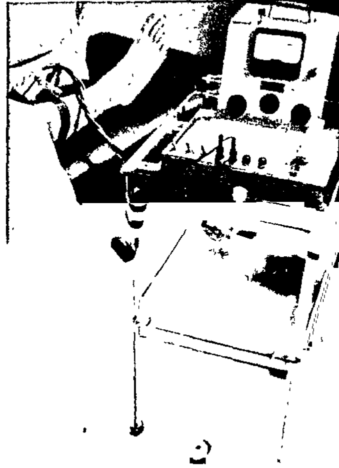


Fig. 30. "Dermofluorograph" for detecting appearance of fluorescein in skin.

mally a small leak for albumin at one spot the resulting extravascular fluid would contain much less albumin per cc. with increasing capillary permeability for water up to the point where suddenly the permeability of the entire membrane is large enough so that albumin can pass through all pores of the membrane. This explains to some extent the low protein content which we find in certain edemas especially in the nephrotic, in the chest fluid, in the ascites and in the peripheral edema.

The next set of experiments consisted of an addition of a second reservoir containing a fluorescein solution (1 mg.%) which can be opened to permit the fluorescein solution to flow into the system instead of the water. Then we introduced a small polyethylene tube into the outer chamber with its open tip just inside of the condom rubber membrane in order to be able to withdraw samples of this "tissue fluid" at given intervals. When the system using plain water was at good equilibrium we shifted from the water reservoir to the fluorescein reservoir and we withdrew samples through the polyethylene tube 1 minute, 2 minutes, 3 minutes and 5 minutes after the start of the flow of the fluorescein solution. The fluorescein content of these samples was then determined in a Fluorophotometer. This experiment was carried out with the candle with the collodion membrane type 1 and then with the candle with the collodion membrane type 2. We found, as we had expected, that the amount of fluorescein contained in the samples withdrawn right at the "skin surface" depended upon the porosity of the collodion membrane; the greater the porosity of the membrane the more rapid the increase in the fluorescein content under the condom rubber surface, i.e., the "skin surface."

That would mean that with increased capillary permeability, fluorescein would appear much faster and in higher concentration at the "skin surface" than when capillary permeability is smaller.

DR. FOX: Suppose you did this instead of with a filter, with hypodermic needles, would you not get the same answer? If instead of the candle you hooked up hypodermic needles, would more fluid flow through the 18 gauge needle than through an 27 gauge needle?

DR. LANGE: Yes, for that one opening there is a change in permeability, for this is what it is actually hundreds of small hypodermic needles.

We then devised an instrument called the Dermofluorograph (Figure 30) which consisted of an incandescent light with a blue filter which throws its light on the skin of the individual. Rigidly aligned to it and fixed together with the light on the skin a phototube is mounted. A yellow filter excludes all blue light from entering and exciting the phototube. That means that if this so-called "search unit" is applied to the normal skin, there will be no current coming from the phototube. When subsequently, fluorescein is given intravenously to the individual and this fluorescein appears in the area to which the search unit is attached, the galvanometer, connected by an amplifier to the phototube, deflects. A strip chart recording of the galvanomemter deflection shows a sudden sharp deflection from the base line. Thus we can determine the length of time (circulation time) it takes for the fluorescein to travel from the point of injection to the point of detection. At the moment of the start of intravenous injection of the fluorescein, a mark is automatically made on the record permitting the exact estimation

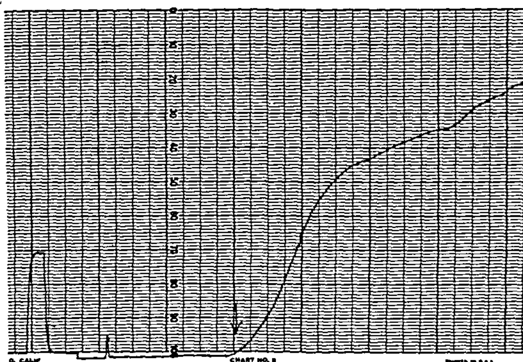


Fig. 31. Normal dermofluorogram showing appearance of fluorosceinbydeflection shown by arrow.

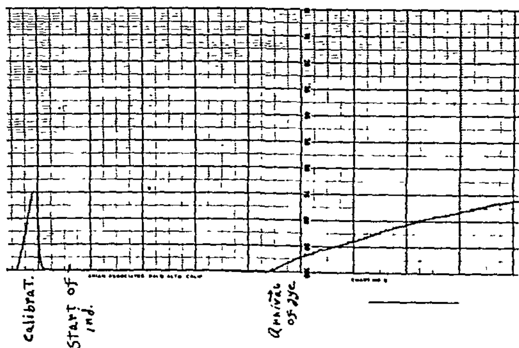


Fig. 32 Dermofluorogram in an individual with peripheral vascular disease and impaired circulation.

of the circulation time by measuring the time interval between the injection mark and the initial deflection. Thus circulation time to any part of the body can be determined automatically in infants, in children and in unconscious persons. Figure 31 shows a dermofluorographic curve of a normal individual taken on the leg while Figure 32 shows a curve from an individual with peripheral vascular disease and impaired circulation. Thus one can establish the normal range for individuals not having any vascular disease and not having any apparent disturbance in capillary permeability provided that the amount of fluorescein injected is kept proportionate to body weight. The shape of the ascent of the curve after the arrival of the fluorescein will depend, as you will understand from our previous discussions mostly upon the capillary permeability provided that the blood supply of the area remains constant. If one takes such curves on an individual with the nephrotic syndrome during the edematous phase one will find that the ascent of the curve is unusually rapid and that after diuresis the shape of the curve returns to normal. While we have at present too few curves of nephrotics during the edematous phase and after diuresis to be of statistical significance it appears that in all of them the ascent of the curve is very steep indicating in our opinion an increased capillary permeability.

DR. RAPOPORT: Is the fluorescein you are measuring in the capillaries diffusing out?

DR. LANGE: Fluorescein is seen only when it diffuses from the vascular bed into the interstitial space but this diffusion is so rapid due to the small molecular weight of the dye that under capillary microscopic observation the appearance of the blood column carrying the fluorescein and its diffusion into interstitial space are noted simultaneously. An exception to this may be the lips where the high concentration of capillaries make the fluorescein noticeable within the vascular tree.

DR. RAPOPORT: How about the factor of spreading of diffusion fluid?

DR. LANGE: This may make a certain degree of difference; hyaluronidase or anti-hyaluronidase seem to accelerate or slow down respectively the spread of the dye when given in high concentration into the test area.

DR. RAPOPORT: The reason I ask this, years ago there was the Aldrich-McClure test which consisted of injecting a tenth of a cc. of salt solution as a wheal into the skin and measuring the rate in which this disappeared from normals. It took about 60 seconds for normal disappearance. In a nephrotic it disappeared almost instantaneously. There were some rather fantastic conclusions drawn from this, but if one injected a wheal of mineral oil this disappeared rapidly in nephrotics. If you put this in a normal it never disappeared. I have got one in my arm for 26 years now. So that actually I don't doubt that water may move back and forth faster in certain situations. Is that what you may be measuring? Is it a fact that things move around in the subcutaneous tissues very quickly once they get out of the capillaries?

DR. LANGE: And unusually quick in the nephrotic.

DR. RAPOPORT: That is right.

DR. LANGE: The presence of excessive amounts of hyaluronidase in the tissue of nephrotics would be a possible explanation of these curves but I feel that this explanation is rather remote.

DR. RAPOPORT: Perhaps that is why they get these subcutaneous spreading infections that don't get marginated, like erysipelas, where excessive spreading factor or lack of something that localizes things prevents margination.

DR. LANGE: On the other hand, you can produce it with any number of counterirritants that you apply to the skin. You would assume that in these cases, too, you would produce something in the skin that makes for fast spreading with heat applied locally, or histamine injected intracutaneously, for example we certainly change the intracutaneous conditions.

DR. LAUSON: I have been interested in Dr. Lange's work for a long time and have raised questions previously. I would like to ask several at this time. Have you overcome the objections I raised that relate to dosage of fluorescein? In other words, the slope of your curve will be a function, among other things, of the plasma concentration of fluorescein. Your original work was based on dosage calculated per kilogram of body weight, which means that, with reduced blood volume and exaggerated body weight due to edema, the plasma concentration in your nephrotic patients should have been a good deal higher than in the normal controls; this alone would increase the slope and raise the curve higher. Secondly, fluorescein is bound to plasma albumin to some extent. I don't know to what extent, but whatever it is, this is a binding that must have among its determinants the plasma albumin concentration; other things being equal, the lower the albumin concentration, the larger the free action of fluorescein. The bound fluorescein is unfilterable through even your most porous postulated capillary wall, and it is only the free dye that is measured at the skin surface. I feel it is essential, in view of these considerations, that the concentration of free dye in arterial plasma should be known at each instant of the observation. Now then, with equivalent arterial concentrations in the nephrotic and the normal, would the nephrotic still show the more rapid and more intensive accumulation of free dye in the skin site? If so, you should continue observations until some kind of equilibrium is established, because *what you now measure is mainly the outward movement from blood to skin tissue* and not much of the return to the circulation of the dye that has filtered out. On the basis of the well-known Aldrich-McClure test and from the various observations of McMasters of the Rockefeller Institute, one might expect that local factors in the skin, other than capillary permeability are involved in your results.

DR. LANGE. We have tried to overcome these objections. The binding capacity of plasma proteins especially albumin for fluorescein remains the same down to a concentration of 1 gr.%. Only at a concentration lower than that does the fluorescein binding capacity of the proteins decrease sharply, and more free fluorescein becomes available. This is very similar to the curve obtained with phenol red. Approximately 70 to 80 per cent of the fluorescein is bound to the protein in plasma and 20 to 30 per cent remains free.

Our nephrotic patients were given fluorescein in amounts figured on the basis of their weight <u>prior</u> to the edematous state.



Fig. 33. Kidneys from uranium injected rat (left), control rat (2nd from left), antiserum injected rat (3rd from left), and rat injected with minimal dose of antiserum (right). Each rat received 2.5 mg. Evans Blue dye intravenously 24 hours before the kidneys were removed.

CHAIRMAN BARNETT: Thank you. I would like to go on now and ask Dr. Goodman if he would tell us about some of the things going on in his laboratory.

D. Dr. Howard Goodman

1. Albumin reabsorption by renal tubule cells in nephrotic rats

DR. GOODMAN: Many of the points I could talk about have already been discussed at some length. I do have one slide which I did not show yesterday because of time. It has to do with a segment of our work in which Dr. Baxter and I injected rabbit anti-rat-kidney serum into rats to produce the familiar nephrotic rats. In addition, we produced proteinuria in other rats by intravenous injection of the smallest amount of uranium (0.1 mg.) which would produce proteinuria. All were 150 gm. female Osborne-Mendel rats.

The rats with proteinuria due to antikidney serum and the rats with proteinuria following uranium injection and control rats all received an intravenous injection of 2.5 mg. Evans Blue dye. Urine was collected overnight, and the rats were killed 24 hours after the dye injection. The kidneys were removed, sliced in half, placed in a Petri dish containing ice-cold saline, and observed under a dissecting microscope. Figure 33 shows a photograph of four of these fresh kidneys. We have made all the usual assumptions which can be criticized and concluded that the appearance of blue dye in the tubule cells of the cortex represents dye-tagged albumin molecules which filtered through the glomerulus and were reabsorbed from the lumen by the tubule cells. Most of the dye is in the form of droplets. On the left is the kidney from the rat with proteinuria due to uranium injection. Due to proteinuria, the rat excreted a blue urine after Evans Blue injection. In spite of the proteinuria, there was no appreciable accumulation of blue dye in the cortex of the kidney. The second from the left is the kidney of the normal control rat which had no proteinuria (and a colorless urine), and at this low dose of Evans Blue, there was very little blue dye in the tubule cells. The third kidney is that of an antiserum-injected nephrotic rat. Here the urine contained protein and was blue, and a tremendous amount of dye has accumulated in the cortex. When frozen sections were examined, much of the dye was in droplets in the convoluted tubule cells. The fourth kidney, on the right, is that of a rat which had developed minimal proteinuria after a small dose of antiserum; the proteinuria had disappeared. Despite the fact that no evident proteinuria remained and the urine was colorless, a few nephrons were definitely blue. We interpret these findings to mean that although dye-labeled albumin was present in the tubule lumen, the uranium-damaged tubules did not reabsorb enough albumin to produce blue-dyed tubule cells. Thus an important disturbance in the uranium injected rat was a decreased tubular reabsorption of protein. I suggested to Dr. Hughes after the meeting yesterday that if these interpretations are correct, uranium might serve to prevent the tubular reabsorption and degradation of serum albumin which is filtered through the glomerulus. He might find this useful in his studies of the relation of the kidneys to albumin catabolism.

In the antiserum injected rat, the glomeruli, presumably damaged by the anti-kidney serum, allowed an increased amount of protein to pass into the tubule lumen. Despite the increased tubular reabsorption of protein resulting in the intensely dyed tubule cells, much of the protein appeared in the urine. In the last kidney (on the right),

a small number of glomeruli were damaged and leaking protein, but the tubules were able to reabsorb all the additional protein, so that none appeared in the urine. If our assumptions are correct, then, in the nephrotic rat there is not a decrease, but an increase in the reabsorption of dye-labeled albumin by the tubule cells..

DR. LAUSON: Or accumulation in the tubule cells.

DR. GOODMAN: Yes, the mechanism for disposal of reabsorbed dye might be disturbed. But one might then expect a similar finding in the tubules damaged by uranium.

DR. FOX: You said leaking glomeruli. Do these indicate any difference in the amount of glomerular leakage?

DR. GOODMAN: If our interpretation of these results is correct. The second kidney from the left is a normal rat kidney with glomerular filtration and tubular reabsorption of a minimal amount of dyed protein whereas the nephrotic rat probably has glomerular filtration of a tremendous amount of the Evans' Blue labeled albumin, in an amount greater than can be reabsorbed by the tubule cells.

The amount of protein in the urine is about 2 mgm. per hour and one could assume with the minimal dose of uranium that the glomeruli had not been damaged, and they do not seem to be damaged by histological sections. What is in the urine could be normally filtered glomerular filtrate protein, which has not been reabsorbed by the damaged tubules. But I would hesitate to use this for evidence about the amount of normally filtered protein, because there is really no proof that these glomeruli are undamaged. They may very well have been damaged by the small dose of uranium.

DR. HEYMANN: Two mgm. of albumin per hour hardly represents a pathological proteinuria in a rat.

DR. GOODMAN: Our rats drink a solution containing 10 per cent glucose overnight and they put out about 50 milliliters of urine during the overnight collection. Thus we are able to do a rather good quantitative estimate of the proteinuria. The normal rat puts out .3 mg. of protein per hour, rarely as much as half a milligram or .6, so anything over 1 mgm. per hour in our 150 gram female rat is significant and these are 2 and 3 mgm. per hour.

DR. HEYMANN: In the nephrotic rat?

DR. GOODMAN: The nephrotic rat will go up to 20, 30, 40 mgm. per hour but this particular one, about 6 mg./hour.

In the uranium injected rat, 2 mgm. per hour so it is 48 mg. in 24 hours. The normal rat is .3 mgm. per hour, or 8 mgm. for 24 hours. This nephrotic rat put out 6 to 8 mgm. protein per hour but as Dr. Heymann pointed out, they go up to 400 mgm. per day. The last kidney on the right was from a rat with no proteinuria, or .3 mgm. per hour.

E. Dr. Norman Kretchmer

1. Work in progress

DR. KRETCHMER: We are primarily interested in the question of amino acid transfer and have been working with it for the past eight years, first with Dr. Jean Oliver and now with the group at New York Hospital. I must say that in the past eight years we have not advanced very far. We do know that amino acids enter the tubule cell, however, this transport is not dependent upon Vitamin B_6 as is the transport of amino acid into the ascites tumor cells. Actually, kidney cells transport amino acids in animals who are extremely Vitamin B_6 deficient as well as in normal animals.

We have been interested recently in tyrosine metabolism. We thought it might be interesting to deal with the analogues and enantiomorphs of tyrosine and see how they differ in their cellular transfer. At the present time we are dealing with p-hydroxy-phenylpyruvic acid and Dr. Etzwiler and Miss Helen McNamara are working on a method for the determination of this substance. We are also doing some work with the sulfated form of tyrosine. In regard to this substance we have found that tyrosine sulfate is not acted upon by the enzymes which usually act on tyrosine. Other preliminary work has been in the use of C^{14} labelled amino acid in protein with renal insufficiency. The purpose of this work is to measure protein turnover.

CHAIRMAN BARNETT: Thank you. Dr. Gribetz, would you say what you are doing down at Mount Sinai?

F. Dr. Donald Gribetz

DR. DONALD GRIBETZ: Our group at Mt. Sinai Hospital has two projects pertinent to the kidney under progress, these are being performed in collaboration with Drs. Jerome Kohn and Avron Sweet and with Dr. Marvin F. Levitt of the Department of Medicine.

1. Bone and tendon as water and electrolyte reservoirs in edema

The first study was designed to test whether or not solid tissues such as bone and tendon would prove to be large reservoirs of water and electrolyte in the presence of edema. Three groups of rats were set up. (a) rats made "nephrotic" by the Heymann technique, (b) rats injected daily with DCA and (c) control animals. Thus far, tendon and bone have failed to yield large amounts of water and electrolyte for the formation of the two types of edema studied. Comparison of the tissue (muscle, bone, tendon and skin) electrolyte composition of the "nephrotic" animals and the DCA injected animals, however, revealed interesting contrasts. In the former, there was simply iso-osmotic retention of salt and water with little or no change in potassium content. In the latter, there was the characteristic hypokalemia with much less salt retention. These data appear to lend support to the doubts concerning whether adrenal salt-retaining hormones of the aldosterone or DCA type are the etiological factors of the edema of the nephrotic syndrome. Perhaps we are not justified in these tentative conclusions; we are continuing the experiments utilizing aldosterone itself.

2. Effect of calcium infusions on urinary electrolyte excretion

The second group of experiments are quite exciting to us since we "stumbled" upon them and thus far we don't understand the full implications. In the course of studying the effect of anabolic steroids on the hypercalcemia of paralytic poliomyelitis, we came across a patient with a low salt-syndrome plus hypokalemia. The following question posed itself: Were the low serum sodium and potassium secondary to mechanical blockage of tubular reabsorption due to the actual deposition of calcium or did the hypercalcuria interfere with some enzyme or other reabsorptive mechanism in more than a mechanical manner? Several members of our group leaned toward the second hypothesis. To test it, we administered acute calcium loads first to monkeys and then to humans (incidentally, everything that Dr. Heymann related about the difficulties of working with monkeys is corroborated). Our results were quite interesting in that within short infusion periods of 20 minutes, hypercalcemia and hypercalciuria stimulated the urinary output of large amounts of sodium and potassium. These data have been consistent under varying conditions, i.e., single injections of calcium, continuous infusions of calcium, etc. In fact, we have succeeded in lowering the sodium and potassium output in the urine by acutely lowering serum calcium. Apparently, therefore, hypercalcemia and/or hypercalciuria will cause an electrolyte diuresis.

DR. LAUSON: Have you measured the filtration rate?

DR. GRIBETZ: Yes. It has been constant.

DR. METCOFF: Didn't A. V. Wolf do this some years ago? I think he has quite a discussion on the influence of intravanous calcium on electrolyte excretion in his book[14].

DR. FOX: How long did the edema prevail before you analyzed the tissue?

DR. GRIBETZ: We attempted to analyze the tissues at the height of the edema. In terms of the anti-rat kidney serum which we used, this proved to be within 1-4 days after the third injection. In our animals, the edematous phase was usually over by the 6th or 7th day after the initial injection.

DR. FOX: There were no differences in the nephrotic edema where the disease lasted longer?

DR. GRIBETZ: No. All we were doing was comparing two types of edema.

G. Other Approaches

CHAIRMAN BARNETT: I might say I am studying the effects of committee meetings on someone interested in nephrosis. However, Dr. Spater, who has recently joined us, has started a program which he might just describe briefly.

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[14] Wolf, A. V., The Urinary Function of the Kidney, Grune & Stratton, Inc., N.Y., 1950.

DR. HERMAN W. SPATER: We are presently in the midst of setting up a new laboratory to carry out some studies of the enzyme systems in renal tubular cells by the use of histochemical techniques. We are especially interested in whether the tubular enzymes studied are influenced by the abnormal proteinuria in the experimental nephrotic rat. Additional studies will also include histochemical and electron microscopic examination of renal cellular elements during the latent period of the duck serum induced disease. It will be of interest to see whether or not structural or functional changes can be detected before the overt evidences of the disease become manifest. Work is now getting under way and we have as yet no data to report.

DR. HEYMANN: Studies of a similar nature have been undertaken by Mary Ellen Hartman from Philadelphia who has histochemical studies on the way. She uses the experimental disease but not in the early stages and not in the latent period induced by duck nephrotoxic serum.

DR. SPATER: Do you know what specific histochemical studies she has undertaken?

DR. HEYMANN: I cannot tell you.

DR. LANGE: I can say that Dr. Wachstein with my group is doing very similar studies. We have not found anything during the first 4 or 5 days. Positive findings occur before the onset of clinical disease. We have not done electron microscopy.

DR. SPATER: Is this work being carried out with the antibody-induced disease?

DR. LANGE: Only the antibody disease (duck serum) was studied.

CHAIRMAN BARNETT: Dr. Metcoff.

DR. METCOFF: I just wonder if anybody has done what we intend to start doing, so that we can save ourselves the trouble of doing it. We first thought it might be worth while to check the often-made statement that the swelling of the glomerular basement membrane is due to the accumulation of polysaccharide. As far as I know no one has measured this. It appears to be a histochemical staining technique and my histochemical friends tell me that there is some question about the precise validity of this technique. We thought we might use the aminonucleoside disease as a means of getting nephrotic glomeruli and then try to isolate the polysaccharide. Perhaps someone has already done this.

Secondly, we are rather curious about the aminonucleoside disease and wonder whether this purine compound does not depend for its action upon some sort of metabolic block. If this is so, where does such a block occur? We hope to explore this avenue.

Finally, we are concerned with the relationship of the cell protoplasm to altered electrolyte composition in the nephrotic syndrome as well as in other situations. We plan to explore the relationship between specific amino acids, certain enzyme systems, and cellular electrolyte exchanges.

DR. HEYMANN: That will be most interesting. Metabolic studies that we had undertaken years ago with the nephrotoxic serum disease in the rat using renal cortex and the Warburg technique revealed that the utilization of glucose and fructose was inhibited, whereas other substrates used did not reveal any abnormality[15] This lesion was noted within hours after the disease had been induced and persisted as long as proteinuria continued. It would be very interesting indeed to know whether the amino-nucleoside disease produces a similar metabolic lesion.

DR. EDER: Do you know about the studies of George Brown at Sloan-Kettering on nucleosides? I think he is studying their effect on purine synthesis.

DR. KRETCHMER: I do not think it was this nucleoside. Apparently the other nucleosides used by Dr. Brown were effective in inhibiting protein synthesis. This may be through a form of competitive inhibition with metabolically necessary nucleic acid precursors.

DR. McCRORY: Dr. Charlotte Liu, in our laboratory, is now working with kidney tissue cultures -- rat, rabbit, and human. We hope to use this approach for studies of the cytotoxicity of anti-kidney antibodies that some people find in humans and others cannot. Dr. Liu has been able to show that rat nephrotoxic serum is cytotoxic for rat kidney tissue cultures. This effect can be neutralized by adding soluble trypsin digest of rat kidney to the nephrotoxic serum. We are now working with the human kidney tissue culture and hope to do similar studies with human sera.

DR. HEYMANN: It is disturbing because the action of the heteronephrotoxic antibodies are highly species specific. The effect noted by the California workers was, however, not species specific.

DR. COOKE: In regard to those studies, are they using the techniques of Puck to get pure cell colonies? I don't know if you are familiar with his work in Colorado. What he has been able to do is to grow pure cell species just as one grows pure cultures of bacteria by having a non-reproducing supportive layer of other cells. I would think such an approach would be very helpful from that standpoint.

[15] Heymann, W., Bueding, E., and Hartman, M. E., Metabolism of Renal Cortex in Renal Cortex in Nephrotic Syndrome in Rats, Proc. of Soc. for Exper. Biology and Med., 79:292, 1952.

IV. COMPLICATIONS AND MANAGEMENT OF THE NEPHROTIC SYNDROME

CHAIRMAN METCOFF: The program this afternoon will have somewhat greater clinical implications than our preceding sessions. The general format of the program will include discussion of complications of therapy of the nephrotic syndrome, the problem and management of the chronic renal insufficiency and the available information gathered in the British Isles, mostly Scotland I believe, on the incidence of the nephrotic syndrome.

Dr. Arneil of Glasgow, Scotland will lead off with some comments on the age incidence of the nephrotic syndrome.

1. Age incidence of nephrosis in Britain and Scotland

DR. GAVIN C. ARNEIL: This discussion of the age incidence of nephrosis in Britain arose from an argument with Dr. Heymann at Copenhagen in which I commented on the apparent preponderance of older children in the U.S.A. series. The slide which is now displayed represents the age at which nephrosis began in 120 cases seen at The Royal Hospital for Sick Children, Glasgow. 118 of these children were seen by two senior physicians, the criteria of diagnosis thus being reasonably constant. They derive from a fairly circumscribed area with a population of at least 2.5 million. Dr. Heymann and I were discussing the peak age of incidence. I think you will observe that the commonest age incidence in this series is around the 12th month of extra uterine life; this peak is maintained during the second year of life but thereafter falls off rapidly. The point of issue is why this peak is at an age approximately twelve months earlier than in the United States. In Britain, and in Glasgow, there has been a feeling that a type of nephrosis occurred in the younger age group which, it was loosely suggested, might derive from mercury ingestion. This was never proven, and I record the idea for what it was worth. Characteristically it seemed to be more evanescent, presenting with rather less edema, less hypercholesterolemia and, in some cases, elevation of the blood urea. The albuminuria tended to clear up in a few weeks and these patients, on the whole, fared well. We have been puzzled to know whether to call such cases nephrosis or not. They appear to have the characteristics of nephrosis, namely albuminuria, high blood cholesterol, low plasma albumin, and edema, and it is difficult to consider them as not being nephrotic. I should very much like to know if anyone can suggest an explanation for this difference in age incidence which appears to be real.

DR. BARNETT: If you divided the patients into two age groups, between birth and 6 months and between 6 months and a year, would most of them be in the latter group?

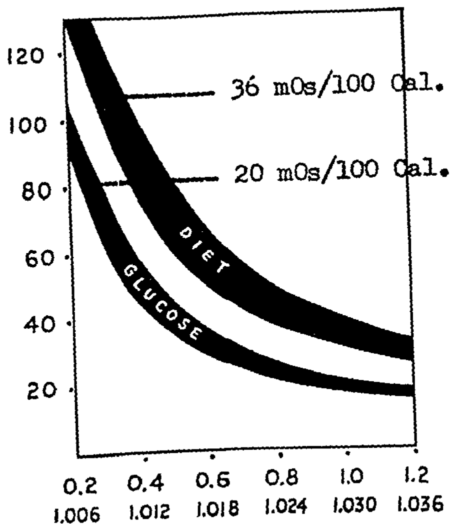


Fig. 34. Relation of urine volume to solute load claiming removal in the urine and to urine concentration.

DR. McCRORY: If you omit neonatal nephrosis, then we have seen nephrosis at 9 months, rarely earlier.

DR. RAPOPORT: And the one child that I remember most vividly I had seen as a newborn and again at 10 months of age in the course of an acute streptococcal infection with purulent otitis. That child developed nephrosis which lasted for three weeks and in another week or two proteinuria completely disappeared with just treatment of the infection. The child is now 11 or 12 years of age.

DR. COOKE: As long as we are discussing cases, I would like to add something. It seems to me the cases of neonatal nephrosis which everyone at the moment is avoiding including in any group may be of great importance as far as studying the pathogenesis of this disease. We were fortunate enough to see this disease in a premature recently. Renal clearance measurements were made at the onset and then as the disease progressed. There were essentially normal inulin and PAH clearances at the beginning of the disease and there was gradual diminution to very low levels with failure to respond to steroid therapy. I understand the child has just died in Connecticut. I think that the study of such cases is most important. I would like to hear Dr. Lange's notion of how this kind of disturbance can be explained in terms of an antigen-antibody reaction, when we have an age group that at least in terms of circulating antibody are very poor producers of antibody, yet the disease characteristically has gotten progressively worse rather than better, as the maternal antibodies are excreted.

DR. LANGE. I don't know, Dr. Cooke. I have not seen any such case. I would like to know was the complement lowered. If antibodies are to be implicated in these cases you should expect a lowering of complement. Maybe they have acquired antibody from the mother, but then as you stated it should get better, not become a vicious cycle where the antigen-antibody reaction promotes new antibody formation, etc. It is difficult to understand offhand. I would have to know more about it.

CHAIRMAN METCOFF: I wonder if Dr. Cooke would be willing to initiate the discussion of that segment of the nephrotic group which goes on to renal insufficiency and presents a very serious problem in management.

2. Some features of management of chronic renal insufficiency

DR. COOKE: I must say that this will sound like a seminar for practitioners after the experimental work that has been presented. I shall discuss only a few features that we have been particularly interested in. I believe that one must visualize this problem of chronic renal failure in terms of a spectrum as far as the physiology of the kidney is concerned -- a spectrum which ranges from that of very marked glomerular insufficiency to the other side of the picture where glomerular insufficiency may be relatively mild and very severe tubular insufficiency predominates. I would like to point out the problems and hope other people with more experience and better ideas can contribute to the discussion. In the discussion of the problem of how to handle the patient with severe renal insufficiency, I think it is worth remembering that one of the most important items that is involved in this management is the regulation of water intake. In this diagram (Figure 34) slightly modified from Darrow's paper, are plotted the amount of water necessary for urine formation per hundred calories metabolized

and urine specific gravity or urine osmolarity. This figure illustrates that in the patient who has an altered capacity to conserve water in relation to solute one must think constantly in terms of the amount of water required for renal function. His requirements for urine formation are relatively narrow -- somewhere in the order of 85 cc. per hundred calories metabolized if his capacity to dilute or concentrate his urine is limited. A high solute load in the diet as in a high protein diet, of course, increases the amount of water needed.

In the patient receiving parenteral fluids the requirement for urine formation will be in the vicinity of 35 cc. per hundred calories since the solute load will be low. In our experience the problem of the water requirement of these patients is one that need not bother us until the patient becomes ill and requires parenteral fluid therapy such as during surgery. Under those circumstances one must keep in mind the important relationship of solute that is actually available for excretion and the patient's capacity to conserve water without solute.

DR. FOX: What does that number on the ordinate mean?

DR. COOKE: 120 milliliters per hundred calories metabolized. The average solute load that his curve was computed from was 20 milli-osmols per hundred calories. This is the amount of solute that is exreted per hundred calories metabolized. This chart shows the amount of water needed to produce a urine for a given specific gravity or osmolarity with that solute load. It does not include the amount of water needed for evaporation or for stool losses. As I indicated before, I think it is important in the management of the patient with renal insufficiency to emphasize the requirements of such a patient during periods of parenteral therapy because under those circumstances the patient does not have ad libitum intake of water and we must predict what his requirements will be. This sort of chart aids in the prediction. If there is very high intake of solute as in some patient who is receiving amino acids or some other material that will lead to urea formation, then obviously more water must be administered than if the patient were simply on a glucose intake, which would spare tissue breakdown and lead to minimal urinary solute load. It is not a problem for the chronic management of the patient as far as I am concerned because it has been demonstrated that it is considerably better for the patient to regulate his own water intake than to attempt to prescribe given amounts of water for that patient each day. In this same diagram one can interpret the problem of nitrogen intake. What do we accomplish or what do we do in the way of harm in the management of the patient with chronic renal insufficiency with a higher and higher nitrogen intake or a lower nitrogen intake. I think there is no question but that the patient with glomerular insufficiency will have a very marked alteration in his non-protein nitrogen concentration in the serum when we markedly increase his protein intake and it is possible to lower the non-protein nitrogen with a marked reduction in the protein intake. What the significance of this is I must say I do not know at the present time. The implications of the usual high protein diet I think are more than simply high nitrogen intake. They generally imply a high phosphate and high sulfate and high potassium intake and I think those aspects may be more important as far as the actual dietary management of this patient than the nitrogen intake alone. As far as the sodium intake is concerned, I think that that is a problem which must be completely individualized. The sodium intake must be individualized not only for a given patient but the amount of sodium that is needed for a given patient must be indi-

vidualized from one week to the next. One patient followed for a number of years showed remarkable variations in his capacity to retain sodium. Again in the management of the patient with chronic renal insufficiency, the sodium intake must be varied to meet the particular state of the patient at the time. For example, there are patients with severe renal insufficiency who are tremendously edematous and become more edematous with a high sodium intake and there are patients who put out large amounts of sodium in the urine and become depleted if not given extra sources of sodium.

I believe we are on a little better ground statistically as far as potassium requirements are concerned in individuals with chronic renal insufficiency. There are certain old rules of thumb which I believe are worth mentioning although exceptions are sure to arise. The study that Elkinton, Tarail and Peters [1] did a number of years ago in which they studied patients with chronic renal insufficiency show a very striking relationship between potassium retention, producing hyperpotassemia (as defined by potassium concentrations greater than 6.5 milliequivalents per liter) through the presence of relative oliguria. They studied 26 patients and of these 26 patients 24 had elevations of potassium. None of the patients who had potassium concentrations over 6.5 had urine volumes greater than 250 milliliters per 24 hour period. All of these patients were adults. In general it is a fairly reliable rule that in the presence of oliguria there is potassium retention and in the presence of polyuria there may be potassium wasting. It is stated fairly unequivocably in some papers, that in the presence of a large urine output the danger of potassium intoxication is minimal or not present on a relatively low potassium intake. We have seen one exception to this. This was a child with measles encephalitis, who was in a respirator, who seemed to have adequate blood pressure, was putting out large volumes of urine and was receiving as intake a milk mixture given by gavage. The general impression was that with a urine output of 1500 cc., this four year old child could not possibly have a rise in serum potassium, however, analysis of the serum revealed fasting concentrations in the order of 9 to 9.5 milliequivalents per liter. There was no evidence of depletion of extracellular fluid volume and sodium concentration was not low. This case illustrates that high urine volumes do not necessarily mean an adequate ability to excrete potassium.

DR. FOX: What was the urine potassium in this situation? Do you remember?

DR. COOKE: As far as concentrations are concerned I cannot say. I know that the intake was that of a milk mixture, yet there was marked hyperpotassemia despite 1500 cc. of urine output each day in a four year old child, which was a surprise to me. A number of workers have shown that as the glomerular filtration rate falls the percentage of filtered potassium that appears in the urine rises. Apropros of the discussion we had yesterday in regard to potassium handling, one can see that if there is a process in the distal tubule in which there is exchange of sodium and potassium and if this process goes on at a fairly constant rate with a marked reduction in filtered potassium, mathematically it would be inevitable that there would be a greater proportion of filtered potassium appearing in the urine. If the filtered potassium decreased enough, one would arrive at a point where there was potassium secretion demonstrable mathematically even though this had been going on physiologically right along. I think that the comments which were made yesterday in regard to the ability of the individual to

[1] Elkinton, J. R., Tarail, R., and Peters, J. P., Transfers of Potassium in Renal Insufficiency. J. C. I., 28.378, 1949.

excrete potassium would have some pertinence as far as the maintenance of the patient with renal failure. It would seem from animal experiments, certainly in rats, and I believe from observations on such patients as the potassium wasters previously mentioned, and also observations on patients with renal failure that the larger the mass of sodium reaching the exchange point the greater the loss of potassium from the body. Again I have to apologize for not having data with me to substantiate these statements but there is ample evidence in the literature. Dr. Alexander Leaf demonstrated a marked decrease in excretion of potassium after the administration of sodium[2].

What is the importance of potassium? We will not discuss the effects of retention of potassium on the heart but that is one of the problems that must be considered in the management of these children. If there is chronic hyperpotassemia it is possible to reduce this markedly by means of dietary restriction of potassium and by providing greater amounts of sodium that may reach the exchange sites. I will not go into the problem of the relationship of potassium concentration to acidosis which I am sure all of you are aware of. One must worry about excessive losses of potassium from the kidney itself under particular circumstances and that this may lead to further renal injury. I am sorry that Dr. Oliver is not here now because I understand that he has carried out microdissections of kidney tubules that have been injured in potassium deficiency and he has found a very specific lesion occurring quite distally in the nephron. It is important, therefore, that when patients require increased amounts of sodium chloride or bicarbonate that one consider the possibility that the kidney tubules do not become depleted of potassium.

DR. KRETCHMER: The lesion of K deficiency is in the collecting tubule where there is vacuolization of cells and new cell types are seen.

DR. COOKE: We have just completed a study and there is evidence that there may be alterations in at least proximal tubular function with potassium deficiency. We have seen the development of aminoaciduria of increased magnitude in the potassium deficient rat. This may mean that it is proximal tubular injury as well as a collecting tubule injury. It may be of some interest to those people who are interested in the relationship of electrolyte to aminoacid metabolism that the findings of the high lysine content in muscle in potassium deficiency demonstrated by Eckel in Cleveland[3] was also seen in the kidney. Analyses of kidneys that we have just finished indicate that there is a rise in the content of lysine in the kidney in potassium deficiency even though there is no obvious alteration in the lysine in the serum and no increased amount of lysine in the urine. Therefore, the kidney and the muscle in that respect are somewhat comparable.

The problem that interested us somewhat in patients with renal insufficiency has been disturbances in phosphorus and calcium metabolism, but I feel inadequate to talk

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[2] Leaf, A., Couter, W. T., and Newburgh, L. H., Some effects of variation in sodium intake and of different sodium salts in normal subjects, J.C.I. 28:1082, 1949.

[3] Eckel, R. E., and Norris, J. E. C., Basic amino acids in experimental potassium deficiency. J. C. I., 34:931, 1955. Also cf. Eckel, R. E., Pope, C. E. and Norris, J. E. C., Arch. Biochem. & Biophys. 52:293, 1954.

about this with Dr. Kramer here. I would like to show a few slides rapidly which illustrate a typical case of nephrosis. This child had onset of his disease at approximately 12 months, received massive albumin therapy, had recurrent edema for 7 years with marked growth arrest beginning at about 17 months of age and complete failure of growth after the age of 2 1/2. He developed progressive renal failure, eventually fractures, bizarre Addisonian-like pigmentation and finally uremic pericarditis. It is of interest that this child's sibling developed nephrosis and apparently has been in complete remission for at least 3 years. Early in the course of the disease the concentrations of calcium and phosphorus were not markedly altered, there was some degree of acidosis and the sodium concentrations remained fairly normal although marked fluctuations in sodium concentration occurred. A striking note is the remarkable variation in the chemical findings with low calcium concentrations being present at times and somewhat later almost normal calcium concentrations, these concentrations varying reciprocally with the phosphorus concentrations. The phosphorus concentration was lowered markedly at times to subnormal levels by administration of aluminum hydroxide. There was one child studied who had phosphorus concentrations of the order of 9 or 10 mg. per cent and after administration of large amounts of aluminum hydroxide (90 cc. per day of amphogel) lowering of the phosphorus to less than 1 mg. per cent was observed in a child whose NPN was well over 150 mg. per cent.

CHAIRMAN METCOFF: There was a two year survival of this patient after the elevation of NPN ranging from 165 to 255 mg.% was observed?

DR. COOKE. Yes.

CHAIRMAN METCOFF: Don't you think that brings up one characteristic point, that chronic renal insufficiency in nephrotic children quite regularly progresses slowly for a year or two until its fatal termination?

DR. COOKE: It can be very slow but I think we could find cases that progressed much more rapidly. The spectrum is just tremendous.

Another remarkable feature which has interested us in regard to chronic renal insufficiency has been the question of the cause of the growth arrest. I am sure all of you are familiar with the recent paper from Cincinnati [4] in which West tried to correlate some parameters of renal function with growth. He concluded that the concentrating ability was reduced and that chronic acidosis was present in almost all patients who did not grow. However he had a large number of patients with a reduced ability to concentrate who had perfectly normal growth, whereas there were no patients with acidosis who had normal growth. He did not believe that there was a factor of inadequate food intake in these cases. The weightage over the height:age ratio in the majority of these cases was greater than .095. The weight was considered to be the dry weight of these patients and this ratio was used to indicate an absence of so-called nutritional hypopituitarism. He did not find a clearcut relationship to azotemia, to chronic infection or to albuminuria.

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[4] West, C. D., and Smith, W. C., An attempt to elucidate the cause of growth retardation in renal disease. A.M.A., Am. J. Dis. Child., 91:460, 1956.

excrete potassium would have some pertinence as far as the maintenance of the patient with renal failure. It would seem from animal experiments, certainly in rats, and I believe from observations on such patients as the potassium wasters previously mentioned, and also observations on patients with renal failure that the larger the mass of sodium reaching the exchange point the greater the loss of potassium from the body. Again I have to apologize for not having data with me to substantiate these statements but there is ample evidence in the literature. Dr. Alexander Leaf demonstrated a marked decrease in excretion of potassium after the administration of sodium[2].

What is the importance of potassium? We will not discuss the effects of retention of potassium on the heart but that is one of the problems that must be considered in the management of these children. If there is chronic hyperpotassemia it is possible to reduce this markedly by means of dietary restriction of potassium and by providing greater amounts of sodium that may reach the exchange sites. I will not go into the problem of the relationship of potassium concentration to acidosis which I am sure all of you are aware of. One must worry about excessive losses of potassium from the kidney itself under particular circumstances and that this may lead to further renal injury. I am sorry that Dr. Oliver is not here now because I understand that he has carried out microdissections of kidney tubules that have been injured in potassium deficiency and he has found a very specific lesion occurring quite distally in the nephron. It is important, therefore, that when patients require increased amounts of sodium chloride or bicarbonate that one consider the possibility that the kidney tubules do not become depleted of potassium.

DR. KRETCHMER: The lesion of K deficiency is in the collecting tubule where there is vacuolization of cells and new cell types are seen.

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The problem that interested us somewhat in patients with renal insufficiency has been disturbances in phosphorus and calcium metabolism, but I feel inadequate to talk

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about this with Dr. Kramer here. I would like to show a few slides rapidly which illustrate a typical case of nephrosis. This child had onset of his disease at approximately 12 months, received massive albumin therapy, had recurrent edema for 7 years with marked growth arrest beginning at about 17 months of age and complete failure of growth after the age of 2 1/2. He developed progressive renal failure, eventually fractures, bizarre Addisonian-like pigmentation and finally uremic pericarditis. It is of interest that this child's sibling developed nephrosis and apparently has been in complete remission for at least 3 years. Early in the course of the disease the concentrations of calcium and phosphorus were not markedly altered, there was some degree of acidosis and the sodium concentrations remained fairly normal although marked fluctuations in sodium concentration occurred. A striking note is the remarkable variation in the chemical findings with low calcium concentrations being present at times and somewhat later almost normal calcium concentrations, these concentrations varying reciprocally with the phosphorus concentrations. The phosphorus concentration was lowered markedly at times to subnormal levels by administration of aluminum hydroxide. There was one child studied who had phosphorus concentrations of the order of 9 or 10 mg. per cent and after administration of large amounts of aluminum hydroxide (90 cc. per day of amphogel) lowering of the phosphorus to less than 1 mg. per cent was observed in a child whose NPN was well over 150 mg. per cent.

CHAIRMAN METCOFF: There was a two year survival of this patient after the elevation of NPN ranging from 165 to 255 mg.% was observed?

DR. COOKE. Yes.

CHAIRMAN METCOFF: Don't you think that brings up one characteristic point, that chronic renal insufficiency in nephrotic children quite regularly progresses slowly for a year or two until its fatal termination?

DR. COOKE: It can be very slow but I think we could find cases that progressed much more rapidly. The spectrum is just tremendous.

Another remarkable feature which has interested us in regard to chronic renal insufficiency has been the question of the cause of the growth arrest. I am sure all of you are familiar with the recent paper from Cincinnati[4] in which West tried to correlate some parameters of renal function with growth. He concluded that the concentrating ability was reduced and that chronic acidosis was present in almost all patients who did not grow. However he had a large number of patients with a reduced ability to concentrate who had perfectly normal growth, whereas there were no patients with acidosis who had normal growth. He did not believe that there was a factor of inadequate food intake in these cases. The weightage over the heightage ratio in the majority of these cases was greater than .095. The weight was considered to be the dry weight of these patients and this ratio was used to indicate an absence of so-called nutritional hypopituitarism. He did not find a clearcut relationship to azotemia, to chronic infection or to albuminuria.

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Several years ago we tried to approach this problem from an experimental standpoint. One of the questions that arose was the activity of the parathyroids in renal failure. After injection of parathyroid extract intravenously, there is an increase in the excretion of phosphorus in the urine in the normal individual.[5]

In patients with renal failure by contrast the per cent of filtered phosphorus that is being reabsorbed is very small. After parathyroid hormones there was no obvious increase in phosphorus excretion and no change in phosphorus reabsorption. The same phenomenon has been demonstrated in patients with acute renal failure and with acute glomerulonephritis. This suggests that the end organ may already be under maximal stimulation and it cannot respond to further stimulation. Whether that interpretation is correct or not I don't know. Experimental studies on the relationship of acidosis and growth have been relatively limited. We have set up a number of experiments in which pair-fed animals were observed in their growth, one of the pair being made severely acidotic by diverse types of procedures. In animals who had unilateral nephrectomy there was no significant retardation of growth between the two groups of animals, the pair-fed controls and the experimentals. The measurement of growth was done by weight and also by measurement of femur lengths. If ammonium chloride is added to the diet of one of each pair of unilateral nephrectomized animals, there is some lowering of the pH, although this is not marked, some lowering of the bicarbonate concentration and some rise in chloride. There was some retardation of growth of the animals on ammonium chloride. Another group of litter mates were divided and 4 per cent ammonium chloride was administered to one group and pair-fed controls were fed the same diet without ammonium chloride. It was impossible to produce a significant acidosis by this means unless 0.2 per cent diamox was added to the feeding mixture. The acidotic animals had pH's of 7.22, the control pH's were 7.46, the bicarbonate concentration of the experimental animals was 17.2 versus 23.9 in the controls. There was hyperchloremia with a chloride of 107 in the experimentals versus 98 in the controls. With 4 per cent chloride and diamox there was definite growth retardation. Another approach to the problem of acidosis was the production of a respiratory acidosis by keeping animals in a chamber with a high CO_2 concentration, approximately 11 to 13 per cent. These animals had a severe acidosis, pH of 7.11, bicarbonate concentration of 50. The growth retardation in the animals with respiratory acidosis who were fed the same quantity of food as the controls was striking. There was essentially no progression of body weight and femur lengths were markedly retarded. When the animals were taken from the chamber with the high CO_2 concentration, there was rapid acceleration of growth on the same food intake as the pair-fed controls. The grams of weight gained per grams of food fed in the animals with respiratory acidosis was .090 and .202 in the animals in the control group, with a standard error of .007, indicating very significant differences. The differences in femur lengths were 2.4 mm. which is a very significant difference.

It would seem obvious from this material that there is something related to or caused by acidosis that interferes with growth. However, acidosis produced in a different way did not lead to growth retardation. When 0.4 per cent diamox was included in

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[5] Kleeman, C. R., and Cooke, R. E., The acute effects of parathyroid hormone on the metabolism of endogenous phosphate. J. of Lab. and Clin. Med. 37:112, 1951.

the diet, animals kept for 18 to 27 days had severe metabolic acidosis throughout this whole experimental period. Animals had pH's of 7.13 compared with controls of 7.36, bicarbonates of 19.8 versus 24.7 and chloride concentrations of 108 versus 99.8. Despite the hyperchloremic acidosis there was no difference whatsoever in the growth of these animals, so that one cannot attribute growth retardation in chronic renal failure to the low pH alone. As far as the acidosis is concerned I think it is important to realize what harmful effects may result from this chronic acidosis. One disturbance that can occur is that further renal injury may result from the hypercalciuria which is found in chronic acidosis in patients with the Lightwood syndrome, for example. For this reason I believe the acidosis should be corrected to prevent the hypercalciuria. Another striking finding is that children with acidosis and severe renal insufficiency may have very marked bone pain and correction of the acidosis has led to decrease in bone pain and improvement in ambulation. The correction of the acidosis obviously has to await the correction of the hyperphospatemia in order to avoid precipitating severe tetany. The correction of the acidosis should be by the administration of sodium and potassium in excess of chloride. How much potassium should be given and how much sodium is relatively minor. The administration of some potassium along with the sodium is important in those patients with minimal glomerular injury and a great deal of tubular injury since this may insure against the production of potassium deficiency and alkalosis with further injury to the tubules of the kidney. As far as other factors are concerned in the management of patients with chronic renal insufficiency, I can state that we have had relatively little experience. I have talked with those who have used cobalt for stimulating red cell formation in these patients and this does not seem to produce any sustained improvement. The treatment of heart failure and the treatment of hypertension are large subjects which I will not go into.

CHAIRMAN METCOFF: Thank you. Would anyone like to comment?

DR. MATEER. Were the rats that were acidotic also anemic?

DR. COOKE: I am embarrassed to say that since we were thinking primarily in terms of electrolyte, we did not do hemoglobins on these animals so I cannot give a definite answer. Rough hematocrits that were done by drawing blood and spinning to obtain the plasma did not indicate any gross difference between the two groups. In fact, the acidotic animals had somewhat higher hematocrits since we were not able to get as much plasma off from given samples of blood.

DR. KRAMER: *In patients whose phosphate excretion failed to respond to the* administration of parathyroid extract was their initial phosphate T_m comparable to the normal or was it diminished? In other words, was there evidence of parathyroid hyperactivity initially?

DR. COOKE: There was a large percentage of filtered phosphorus rejected by the tubules prior to the injection of parathyroid. Only 26 per cent of the filtered load was reabsorbed in patients with renal insufficiency versus 96 per cent or so in the control.

DR. BERGLUND. Could you review the known actions of the parathyroid on the renal handling of phosphate? The literature is confusing on this point.

DR. COOKE: In a paper written by Kleeman and myself we thought we had pretty good evidence for alterations in the reabsorption of phosphorus after intravenous injection of parathormone. I think these studies had the defect of all studies that measure glomerular filtration rate: that little changes in filtration rate would have made big changes in our calculations. On the intramuscular administration of parathyroid hormone, I think there is fair agreement that this may alter the handling of phosphate by the tubule without immediate increases in glomerular filtration rate, but we did not observe this.

DR. BERGLUND: Does it increase the excretion in normal subjects?

DR. COOKE: It will increase the excretion in normals, if you have a preparation that is active. The difficulty is that many of the preparations are inactive. You have to have a control in your laboratory that is willing to be injected with some of the parathormone to test the hormone activity. There is a difference between the intravenous injection and the intramuscular injection, in that a number of people, especially those working with dogs, have shown that the glomerular filtration rate increases after the injection of parathyroid hormone, and they explain the increased phosphorus excretion in those terms. We did not see this and it appears to me that the literature is about evenly divided.

DR. BARNETT: There is a recent paper by Calcagno in which he studied the effect of different routes of administration and measured the glomerular filtration rate. I don't think the problem is entirely settled. I believe that that paper does clarify some of the details. It was in Pediatrics about three months ago.

DR. BERGLUND: What kind of parathyroid extract did you use? Was it U.S.P. Parathyroid? My reason for asking is Munson's statement [6] that more recent preparations of parathyroid extracts have much less effect on the kidneys and more direct bone effect as compared with the extracts tested some decades earlier. Munson suggested the existence of two separate factors in the parathyroid glands.

DR. GRIBETZ: The only preparation which is available on the market now is the one made by Lilly, except for Munson, who makes his own and there is no question apparently as you look through the literature that there has been a decreasing effect of this Lilly preparation on the kidney. At least the papers seem to indicate this over the past few years.

DR. BARNETT: I don't think any of the kidney people have ever denied it, and Albright made the statement one time that there was probably a second effect of the parathyroid hormone directly on bone.

DR. LANGE: I have a question to ask. Why does a patient in renal failure excrete a 1.010 specific gravity urine anyway? Why does he not excrete two or three times as many metabolites when we give him four quarts instead of two or three since specific gravity is "fixed"? The water seems to be excreted. The average uremic is dry. He can handle water perfectly well.

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[6] Munson, Annals of the New York Academy of Sciences, 60:776, 1955.

DR. COOKE: I think that one of the points that the first figure indicated was that there may be difficulties in excreting water in patients who are unable to dilute the urine. Would you not agree that there are patients in whom the administration of large amounts of water may actually precipitate water intoxication? I am not in agreement that these patients should be loaded with water because some of them at least will not be able to dilute their urine markedly and if they have just so much solute to excrete which is mainly dependent upon the diet then that excess water will be retained in the body.

DR. LANGE: I want to look at it the other way. The patient has a retention of metabolites due to inablity of the kidney to excrete them. Now you give more water to remove them. Now he has more vehicles to carry the solute and the vehicles are half loaded. Isn't it a better situation than to have only half the number of vehicles to carry the load?

DR. SHIPP: Can you actually increase the filtration rate?

DR. COOKE: No. The question Dr. Lange raises is can you alter the back diffusion by keeping the concentration of urea or these other products lower if there is a greater volume of water? I would think theoretically there might be slight advantage to that but there is the risk of not mobilizing solute and having water intoxication as the result. Certainly you see this in the surgical patients all the time who ought to be able to put out a dilute urine but don't.

DR. LANGE: We have never studied why human beings maintain a fixed 1.010 specific gravity whether they receive one liter or two liters. Perhaps they are better off receiving three liters than one.

DR. FOX. They keep on excreting electrolyte at fairly uniform concentration all the time, so, as Bob points out, they reduce the total electrolytes in the body and get water intoxication. They constantly excrete electrolyte at the same level. If you ingest a quart of water you put out a thousand cc. with almost no electrolyte. The patient with advanced renal disease will put 50-60 meq. of potassium in the urine, whether he takes a lot of water or less.

DR. LANGE: Does he not have more urea?

DR. BARNETT: You don't increase the rate of urea excretion very much by increasing urine flow.

DR. KAPLAN: You are afraid of giving alkali because of the danger of tetany. Do I take it then that you advocate a citrate solution such as the Albright mixture?

DR. COOKE: The first step is the correction of the hyperphosphatemia to avoid the development of tetany and the second step is the correction of the acidosis. But I think that we have to go in that order. If a patient has a phosphorus of 12 in our experience at least, when we have tried to correct his acidosis, he has immediately gone into tetany and we have to lower the phosphorus by means of diet and by aluminum hydroxide and then go ahead with the correction of his acidosis. I would like to ask Dr.

Kramer whether or not there is any advantage in citrate, for example, over acetate. It all becomes potential bicarbonate when metabolized. Do we transport into the body an increased amount of calcium by giving our alkali as citrate rather than as acetate?

DR. KRAMER: I never tried acetate. I only used the citrate.

DR. COOKE: Citrate produces diarrhea in children and we have not noted that acetate did this as much.

DOCTOR: How much amphogel do you use?

DR. COOKE: We varied it some with the size of the child. I may say that I have been overenthusiastic in this therapy and that is why the phosphorus levels may go from 10 to 0.9 mg. per cent. We have used as much as 90 cc. a day in a patient of approximately three years of age. In practical terms the mixture that we used was that recommended by Shorr and consists of 10 grams of calcium lactate suspended in 100 cc. of amphogel. One of the dangers which one must be aware of with such therapy is that extreme hypophospotemia can be produced. I do not know what the significance of this is. I would think that it might be like rickets induced in the rat. It is probably not beneficial to the patient.

DR. McCRORY: Was your patient taking amphogel when rickets developed? One effect of amphogel is to prevent the absorption of phosphate from the G.I. tract. If enough is given, phosphate depletion can be marked and rickets could easily develop in addition to the hyperparathyroid demineralization.

DR. COOKE: I thought we were producing rickets in one little girl who had quite marked bone changes. However, before we embarked on any amphogel therapy, she had a high phosphorus level with very definite bone changes by X-ray. With the administration of amphogel her serum phosphorus fell markedly. I think there is one interesting point in regard to the acidosis in these patients, namely, that frequently this is hyperchloremic acidosis and not due simply to higher phosphorus or sulfate concentrations. The phosphorus concentration may fall with amphogel therapy and the bicarbonate in our experience will rise considerably more than simply that number of milliequivalents of phosphorus. Why this occurs I am not at all sure. I do not think, therefore, that rickets is necessarily related to the limitation of the phosphorus, but it might possibly make it worse. This patient had severe bone pain, the little girl I am referring to, and with the correction of her acidosis the bone pain disappeared and she was able to be very active.

DR. BARNETT: We have seen one instance of the most remarkable healing of renal rickets associated with just giving amphogel and calcium lactate. I think if you carry it to an extreme it could do what you say. Early in the course of our patient, there was remarkable healing of renal rickets with lowering of serum phosphorus and he did have elevation of serum calcium with this.

DR. COOKE: Did the acidosis become corrected at the same time?

DR. BARNET: Yes.

DR. COOKE: Sections of bones of animals made acidotic show typical osteitis fibrosa.

DR. GRIBETZ: Are we right in assuming that you ascribe growth failure in chronic renal insufficiency to occur prior to changes in calcium-phosphorus metabolism and bone changes, evn though they be subclinical?

DR. COOKE: I was not trying to ascribe growth failure to any one factor because I am puzzled as to why these children do not grow. I do not believe from the studies that were shown on the board, particularly the chronic acidosis experiment in which *diamox was administered, that acidosis per se need interfere with growth. Is there a* difference in the acidosis of the rat receiving diamox and the patient with this sort of kidney disease? I think this question may be most critical. The animal on diamox has a precipitous increase in the excretion of sodium only for the first day or two of the administration of diamox and then the sodium excretion returns to the same level as before, the only difference from that time on between this animal and the control is that there is a low pH, a high chloride and low bicarbonate. That seems to me to be a different situation from the animal who is receiving a constant load of some other material such as ammonium chloride that may be bringing sodium or calcium or something else out of bone. I feel that one should not throw out the possibility that the changes that go on concurrently with the acidosis may not be responsible for the growth arrest. I am skeptical that it is the pH change alone.

CHAIRMAN METCOFF: I think we will go on. Dr. Mateer will present another aspect of the management of chronic renal insufficiency, through vivo-dialysis.

3. Hemodialysis in chronic renal insufficiency

DR. MATEER: Hemodialysis has usually been considered an adjunct to therapy of acute renal failure. However, the possibility of a reversible lesion superimposed upon chronic renal insufficiency simulating terminal renal failure has led us and others[7, 8] to use of dialysis in patients otherwise thought to be unsuitable for the procedure.

In 35 apparently terminal uremic patients 30 successful dialyses were performed. Three of the other 5 died of potassium intoxication while dialysis was being started. One other patient died of a cerebrovascular accident with primary respiratory arrest after 40 minutes of dialysis. The fifth patient, a 32-year-old veteran with unilateral nephrectomy and pyelonephritis, died of a cardiac arrythmia attributed to potassium deficiency after 8 hours of dialysis despite inclusion of potassium in the dialysing bath as soon as a change in cardiac mechanism was detected.

Of the remaining 30, ranging in age from 11 to 55 years, 10 had pyelonephritis, 12 glomerulonephritis, 2 nephrosclerosis, 4 diabetic nephropathy, one disseminated

[7] Goldner, F., Gordon, G. L., and Danzig, L. E., Use of Artificial Kidney in Chronic Renal Disease, Arch. Int. Med., 93:61, 1954.
[8] Mateer, F. M., Greenman, L., and Danowski, T. S., Hemodialysis of the Uremic Child, Am. J. Dis. Child. 89:645, 1955.

lupus erythematosus, and one renal tubular acidosis. All of these patients appeared to be in a terminal uremic state. Acidosis was invariably present. Central nervous system abnormalities such as convulsions, stupor or coma were frequently present as was pulmonary edema and congestive failure. Potassium intoxication was noted in only three. It is of interest that one patient who later survived 18 months had a pericardial friction rub and a toxic psychosis manifested by hallucinations at the time of dialysis.

The dialyser used in all instances was a Westinghouse modification of the Alwall type[8, 9]. This consists of a set of stationary rigid metal screens in the form of cylinders wound with a dialysing tubing flattened by a wire grid jacket to provide greatest surface area for least blood volume. The length of cellophane tubing, 1 1/8 inches wide when flattened, wound upon the screens is either 50 feet or 100 feet. This is immersed in a 32 liter bath kept at body temperature. The bath fluid contains 120 gm. sodium chloride, 60 gm. sodium bicarbonate, and 50 gm. glucose dissolved in a partially full 5 gallon carboy of tap water. Carbon dioxide is then bubbled through the solution for 5 minutes and 4 gr. of calcium chloride (as 10 percent solution) are added, and the solution made up to 5 gallons. The resulting solute concentrations are: sodium 142 mEq/L, chloride 107 mEq/L, bicarbonate 35 mEq/L, calcium 7 mg/100 ml. and glucose 255 mg. 100/ml. In patients without anuria 4 mEq/L of potassium chloride is included in the bath fluid. Bath change is effected every 200 minutes.

The mean amounts of solute added or removed from a patient in the course of a dialysis corrected to body weight of 70 kilo are tabulated in Table 12. The mean change in blood and serum constituents is also shown.

TABLE 12

Solute added (+) or removed (-) in the course of 30 hemodialyses
(mean values corrected to 70 kilo)

NPN	K	Na	Ca	Cl	PO_4
- 55.8 gm.	- 66 mEq	+ 14 mEq	+ 304 mg.	+ 119 mEq.	- 2.28 gm.

Mean changes in blood and serum concentrations in the course of 30 hemodialyses

NPN	HCO_3^-	Cl^-	Na^+	K^+	Ca	PO_4
mg/100 ml	mEq	mEq	mEq	mEq/L	mg/100 ml	mg/100 ml
- 85	+ 6.4	+ 6.9	+ 10	- 3.2	+ 1.4	- 5.7

[9] Elkinton, J. R., and Danowski, T. S., The Body Fluids: Basic Physiology and Practical Therapeutics. The Williams and Wilkins Company, Baltimore, 1955. Chapter 25, pp. 549,568.

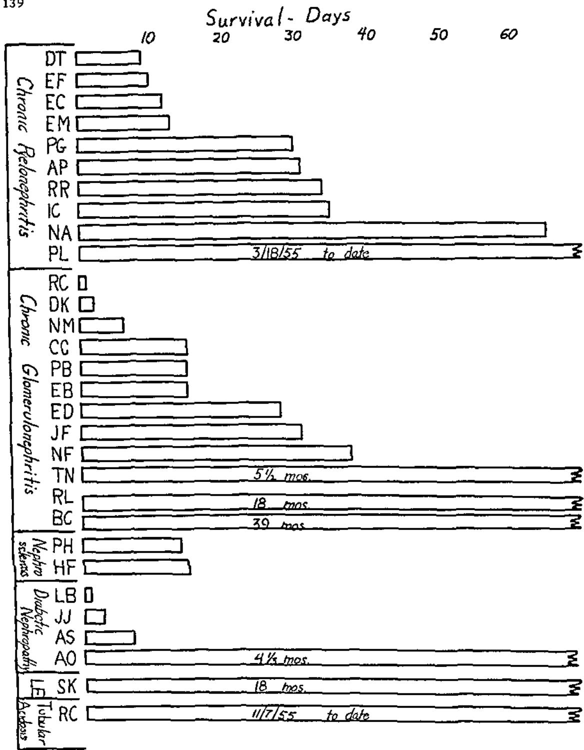


Fig. 35. Duration of survival of dialysed patients with chronic renal failure.

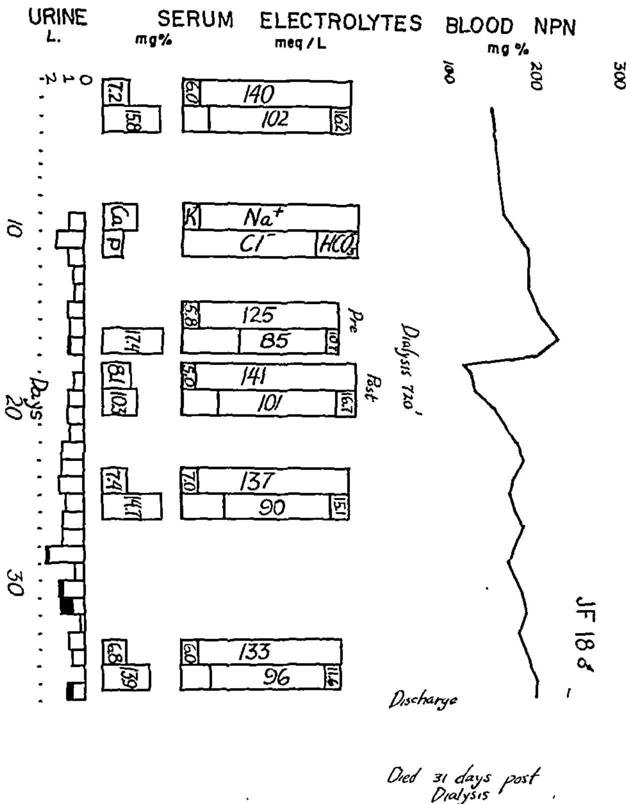


Fig. 36. Blood and serum constituents observed in a patient with chronic glomerulonephritis pre- and post-dialysis. This 18 year old schoolboy had acute glomerulonephritis five years before admission. There was stupor, edema, and severe vomiting which improved sufficiently after dialysis so that oral intake could be resumed.

DR. LANGE: These values are corrected for the blood given?

DR. MATEER: They are not. The exchange transfusion effect of blood used in priming the machine produces slight immediate changes as reflected in blood samples taken at a 20-minute interval but has little effect on total solute transfers. The average change in blood and serum concentrations are initial minus final values.

The clinical effects are invariably salutary, the patients become more comfortable, edema is lessened (body weight diminishes 1 to 3 kilograms), central nervous system symptoms are reduced. In many instances oral intake precluded by vomiting previously again becomes feasible.

In this group of apparently terminal renal failure, 15 lived more than 3 weeks, 8 more than 2 months and 2 are still living (Figure 35).

DR. RAPOPORT: How many children do you have in that series?

DR. MATEER. Since the experience with 5 patients previously published[8] we have dialyzed 3 additional children, one an infant. Three are included in this chart.

The following case illustrates in more detail some of the effects of hemodialysis. J.F. (Figure 36) was an 18-year-old boy with glomerulonephritis of 5 years' duration, hospitalized with an apparent acute exacerbation of the disease. The clinical deterioration paralled the rise in NPN, development of hyponatremia, an increase in acidosis with phosphate retention and a striking rise in the undetermined anion fraction. After 720 minutes of dialysis he lost 2 kilograms of body weight, the sodium concentration was restored to normal, and the acidosis decreased. Clinical improvement was definite in that edema was reduced, oral intake resumed, and central nervous system function improved. However, gradual deterioration occurred and the patient died 31 days post-dialysis.

A particularly rewarding result was obtained in patient P.L., a 23-year-old woman with acute and chronic pyelonephritis in the eighth month of pregnancy. After development of uremia, she improved sufficiently under medical management, a Caesarean section was performed and a living baby obtained. However, on the eighth post-operative day she convulsed. At this time edema and hyponatremia were present. Dialysis corrected the hyponatremia with sodium concentrations rising from 125 to 140 mEq/L, and edema was concomitantly reduced. The patient recovered, though chronic pylonephritis with azotemia (50-70 mg/100 ml) persisted 20 months later.

Superimposition of urinary tract infection in a patient with glomerulonephritis may require the aid of dialysis to restore a patient's precarious compensation. This is illustrated by our experience with B.C., a 15-year-old boy with a 3 1/2 year history of a nephrotic syndrome treated on two occasions with a 28-day course of ACTH and progressive renal failure. He was admitted with vomiting, diarrhea, progressive acidosis, pulmonary edema and convulsions. The whole blood NPN was 195 mg/100 ml, serum sodium 135 mEq/L, bicarbonate 5 mEq/L, phosphate 13 mg/100 ml, and calcium 4 mg/100 ml. After dialysis the convulsions stopped, NPN was 85 mg/100 ml, sodium concentration was 140 mEq/L, bicarbonate 16 mEq/L, phosphate 6 mg/100 ml, and

calcium 7 mg/100 ml. A marked pyuria due to cystitis improved with therapy and the patient was discharged. He subsequently died in uremia 14 months later.

Feasibility of repetitive dialysis in maintenance of a patient with practically no renal function has been explored in patient N.F., a 19-year-old girl with chronic glomerulonephritis admitted with NPN of 253, and daily urine output of less than 200 ml. By *weekly dialyses, four in all, it was possible to keep this patient alive for 38* days. Feasibility of additional dialysis was precluded by lack of available blood vessels for cannulation.

In summary, the most hopeful use of dialysis would be in therapy of acute load superimposed upon chronic disease, whether it be systemic infection, urinary tract infection, pregnancy, or temporary exacerbation of the primary disease process. Specific benefits include improvement of hyperkalemia, acidosis, hyperphosphatemia and hypocalcemia. By ultrafiltration, fluid excesses can be reduced. Clinical improve*ment in pulmonary edema, and central nervous system changes are definite. Sympto*matic effects in excess of objective findings are frequently observed. As dialysis becomes a simpler procedure, it may provide a more effective tool in therapy of chronic renal disease.

CHAIRMAN METCOFF: Thank you. Are there any comments?

DR. BERGLUND: How do you control the blood flow? By the patient's own pressure?

DR. MATEER: Flow is controlled by a roller pump which pumps equal amounts of blood into and out of the dialyser, keeping the volume constant. Despite heparinization bleeding has been a problem in only two patients, both postpartum, who had uterine bleeding.

DR. HEYMANN: How about the factor of post-dialysis anuria which is possibly due to the decrease of urea concentration?

DR. MATEER: Oliguria has occurred after dialysis, but not anuria.

DR. HEYMANN: Has it ever been threatening or disturbing, or is it not so bad? Does oliguria stop very soon? How long does it last?

DR. MATEER: Resumption of predialysis urine volumes has usually occurred within two or three days of dialysis. This has not been a serious consideration in dialysis.

DR. BLAINEY: Are the patients very anemic and does the restoration of blood volume play a part?

DR. MATEER: Because the dialyser is primed before connection to the patient, no net change in blood volume occurs. There is no attempt to correct anemia during dialysis. After dialysis transfusion is often given, inasmuch as danger of congestive failure and pulmonary edema has been lessened.

CHAIRMAN METCOFF: Some years ago when Dr. John Merrill was getting started with his experiments with vivo dialysis, it seemed possible that some instances of the nephrotic syndrome occurring in children might be benefited by dialysis. For example, those patients might be good candidates for dialysis in whom significant, severe renal damage was not evident, but who had acquired acute renal insufficiency as a result of some acute insult, such as severe infection. In one instance the dialysis was successful, but unfortunately, the patient subsequently died of pancreatic necrosis a few days following dialysis. In two other instances that I recall, chronic renal insufficiency was treated effectively by dialysis with temporary improvement, but the patients ultimately succumbed to recurrent renal insufficiency. In one of these instances, a rather interesting physiologic lesson was learned from a child of about four years of age with uremia. The Kolff type kidney was used for dialysis. This machine requires priming with about a liter of blood. This child was on a ward where most of the patients had leukemia or tumors and it was necessary for the house officer to transfuse almost every patient each night. Blood was obtained the evening prior to dialysis for this child, who was in mild congestive failure, with typical uremia and hypertension. The blood was discovered in the icebox by the house officer about midnight and he felt that he had committed a serious error by not having transfused this child earlier. Since the blood had been sitting there for some time, he thereupon proceeded to infuse a liter of blood into this four year old child. The infusion was completed by the next morning. That child was a most rosy cheeked youngster when we saw him in the morning. He suffered no inconvenience. This was a remarkable example, it seemed to me, of the resiliency of the vascular system in young children, and probably another indication of the difference between children and adults with chronic renal insufficiency.

DR. BARNETT: This raises a point that I want to bring up: I know Con Riley has been impressed for a long time with the frequency with which bad events follow transfusions in children with chronic renal insufficiency, even when given in small amounts and very carefully. I must say this is not uncommon in our experience. I am very leery of transfusing a child with severe chronic renal insufficiency and I don't know exactly what happens. It is not pulmonary edema or signs of overexpansion of blood volume but to have a child convulse or die within a day or two after a transfusion has been all too common in our experience. Maybe it is because we finally are driven to transfusion at a time when they are going to die anyway. But I would like to hear other people's experiences about this.

DR. HEYMANN: We had nobody die but have had one convulsion.

DR. McCRORY: A different problem arose with a patient we observed. One girl who was followed for five years with marked renal insufficiency was transfused when hemoglobin levels fell below 7 gm.%. She required transfusions because of fatigue and nosebleeds. We decided to see whether intervals between transfusions could be prolonged or patient improvement observed by maintaining a more nearly normal hemoglobin level. We accordingly tried to keep her hemoglobin around 10-12 gm.%. The patient became more hypertensive, had headaches, and more frequent epistaxis on this improved regime. Consequently, we went back to minimal transfusions.

CHAIRMAN METCOFF: I hope it is clearly understood that I am not advocating our unusual experience as a means of therapy.

DR. LANGE: We had the same experience. We don't transfuse unless we absolutely have to. We feel it is a little bit better to take fresh blood from donors. I think bank blood has something to do with it. Since we have done that the last two years we have not had trouble. We don't like to transfuse in large amounts but only in small amounts, 250 cc. as a maximum.

CHAIRMAN METCOFF: Would anybody care to comment about citrate as a blood preservative?

DR. HARRIET G. GUILD: In recent years, we have used only fresh blood and have separated the cells from the serum, giving packed cells rather than whole blood to these patients, in amounts not exceeding 50-75 cc. at a time. This not only gets rid of the citrate, but also reduces the volume that has to be given for a given level of hemoglobin. We have thought that, in removing the serum, we have disposed, also, of still other factors in the donor's blood that may be responsible for unfavorable reactions. Occasionally, in cases of advanced chronic nephritis with severe hypertension, we have given, instead of packed cells, daily injections of 20 cc. of whole blood, removed directly from the donor (usually a member of the staff!) at the bedside, without the need for the addition of citrate or other preservative. Since exercising these precautions, we have had no difficulty in transfusing patients with chronic nephritis, whereas, previously, we had encountered the problems referred to by Dr. Barnett.

DR. COOKE: Citrate? Is there evidence that it is not as quickly metabolized in the nephrotic as in the normal?

CHAIRMAN METCOFF: There is some evidence that the citrate contained in blood bottles as an anticoagulant predisposes to hypocalcemia and possibly tetany[10].

DR. COOKE: *If the material is given slowly,* I thought the exchange transfusion data showed very clearly that the amount of citrate that persists in the circulation is negligible.

DR. MATEER: With respect to anemia, we have seen a girl of 15, with chronic glomerulonephritis of 5 years duration, who asked at her last clinic visit whether she could go swimming. Her hemoglobin at the time was 6 gm., and she had missed but three days of school the previous year. Our policy has been to transfuse only if symptoms appear and, arbitrarily, only to a level of 10 gm. of hemoglobin.

DR. ARNEIL. I am interested in this. One of the factors we have noted in stored plasma is a marked pressor activity, of unidentified origin, which is not present in fresh plasma.

DR. BARNETT: This may be important.

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[10] Nakasone, N., Watkins, E. Jr., Janeway, D. A. and Gross, R. E., Experimental studies of circulatory derangement following the massive transfusions of citrated blood. J. Lab. Clin. Med. 43:184, 1954.

CHAIRMAN METCOFF: Dr. Fox, would you care to comment?

4. Acidosis in chronic renal insufficiency

DR. FOX: Some years ago we were interested in this problem of chronic renal insuffiency and I was surprised to see the tremendous amount of literature that is available that supports many of the points that you brought out, Bob. One of the publications that Dr. Van Slyke told me about, which I had not seen, which might be worth noting is the fine monograph by Kirk[11]. Kirk, at the Rockefeller Institute, conducted an extensive study of acidosis. Hartman has shown if one corrects an acidosis there is a concomitant fall in plasma urea. The curve of rising plasma bicarbonate is followed by the curve of falling urea.

Recently Stewart[12] informed me of his earlier studies, suggesting that protein diets which result in acid formation, are deleterious compared to equivalent protein diets which are basic. Chronic renal insufficiency may represent a situation where administration of sodium as chloride has different effects and is less beneficial than sodium administered as acetate or lactate, i.e., with a metabolizable anion. One of the problems, apparently, is renal excretion of anionic radicals. These can be excreted with sodium or potassium when these cations are administered with metabolizable anions which do not require renal excretion as does the chloride. Further evidence for a rational basis for the treatment of chronic renal insufficiency with alkali is cited by Jean Oliver[13]. There was abundant evidence to show that precipitation of solid material in the tubules is favored by an acid urine and that much of the occluding material is soluble in alkaline urine. Experimentally, the appearance of solid matter in acid urine as casts and their disappearance in alkaline urine, has been demonstrated. Considerable discussion of the factors responsible for coagulation and cast formation is given in his book and in his Harvey Lecture[14]. This lecture also gives information regarding the protein content, pH and total electrolyte in the urine in causing coagulation in the tubules.

In this connection, insufficient attention has been paid to the electrolyte composition of the urine. In Table 13 are shown the changing electrolyte patterns of the urine of acute renal failure following a mismatched transfusion. During the interval that the patient became increasingly oliguric, the electrolyte concentrations in the urine resemble that of a glomerular filtrate, as shown in days 1-5. During this time the blood urea nitrogen rose rapidly. On the eighth day and subsequently as the renal failure was relieved, the urine sodium decreased and the urine potassium increased. Patients with chronic renal failure usually form urine containing 50-55 mEq/L of sodium and potas-

[11] Kirk, E., Acidosis, - Clinical Aspects and Treatment with Isotonic Sodium Bicarbonate Solution. William Heineman Medical Books, Ltd., London, 1946.

[12] Lyon, D., Dunlop, D., Stewart, C. P., Effect of Acidic and Basic Diets in Chronic Nephritis. Edinburgh Medical Journal, p. 87, February, 1931.

[13] Oliver, J., Architecture of the Kidney in Chronic Bright's Disease. Paul B. Hoeber, Inc. Medical Book Dept. of Harper and Bros., N.Y., 1939.

[14] Oliver, J., New Directions in Renal Morphology: A method, its results and its future. Harvey Lectures, XL, p. 102, 1944-45.

sium. The urine concentrations of these patients are monotonously uniform. This is true whether large amounts of water or large amounts of electrolyte are administered. Increased excretion is usually accomplished by increased total urine volume. This is one of the reasons why administration of large amounts of hypertonic electrolyte intravenously is probably hazardous and should perhaps be avoided.

TABLE 13

Urine in Renal Failure

Day	Volume cc	Na	K	Plasma K
		mEq/Liter		
1	145	120	12	4.4
3	44	120	12	
5	90	125	10	4.9
8	480	109	24	6.7
9	815	88	32	
11	981	52	65	7.9
13	1640	49	48	
14	800	42	47	4.9
20	1200	28	46	4.2

Acidosis, however, can be corrected fairly readily by oral administration of alkali. One should remember that chronic renal acidosis usually occurs slowly over a long period of time, hence correction should be accomplished gradually and preferably by daily administration of sodium acetate or lactate, by mouth.

Table 14 concerns a man with little kidney function left: one kidney had been removed and the remaining kidney was hydronephrotic with a large calculus. Like so many patients with elevated blood urea nitrogen, his CO_2 was extremely low and urine volume was small. He was given orally, one molar sodium acetate solution. I should emphasize that a one molar solution given orally is diluted by other liquids and food; thus a patient can absorb 100-200 mEq a day by ingesting 100-200 cc. of this solution. With this therapy, the blood urea nitrogen (BUN) goes down as the CO_2 goes up. The urine volume is augmented and the total nitrogen excretion increases considerably. Apparently more urea can be excreted in a urine containing electrolytes than in a urine practically devoid of electrolytes. A study by Gamble [15] showed that urea "fits" into urine with other solutes. Water expenditure in the presence of urea was found to be much less than the sum of the water requirements as determined separately for urea and electrolytes. Dilution of the body fluid with water does not induce elimination of as much urea as does administration of sodium and potassium salts to overcome acidosis and achieve isotonicity. Urine with sodium and potassium approximating 50 mEq per liter of each is obtained. Plasma sodium when subnormal can sometimes

[15] Gamble, J. L., et al., An Economy of Water in Renal Function Referable to Urea. Am. J. Physiol. 109:139, 1934.

be elevated to normal and most of the sodium is excreted. It is amazing that following administration by mouth in divided doses of large amounts of alkaline sodium salts to patients with poor renal function, most of the administered sodium is excreted and concomitantly the blood urea nitrogen is reduced.

TABLE 14

Relief of Azotemia and Acidosis by Sodium Acetate

Single Kidney with Calculus Hydronephrosis

Day	BUN mg%	CO_2 mEq/L	Urine 24 Hr. cc.	Urine Total N_2 g.	Therapy
					F
					L
1	--	--	00		U
2	59	--	176		I
6	172	6.5	1300	--	D
7 AM	181	5.3*	400+	--	S
	One Molar Na Acetate Orally				cc.
7 PM	191	18.0	2000	--	500
8	---	--	2100	11	300
9	180	27.0	3800	22	200
10	---	--	3520	20	180
11	120	26.6	2750	17	100
12	84	26.6	2450	16	0
13	72	19.0	1800	--	K Acetate
18	34	24.0	2800	--	10 gr.

*Plasma Na 122$\rightarrow$133, 80% Na excreted in 6 days.

We have tried to conduct some calcium studies but do not have enough data to present; these patients received 10-20 grams of calcium lactate daily. Some are given amphogel. Their water intake is increased only insofar as they increase it by ingestion; there is a greater tendency for patients who have been receiving sodium and potassium salts to drink more water. If a lot of water without electrolyte is given, difficulties arise because of their inability to dilute the urine like the normal individual does. A large increase in urine flow can be achieved by a greater water intake if enough electrolyte is given also to maintain urine sodium at about 50-60 mEq per liter without further withdrawing sodium from the body. Thus, to obtain two liters of urine, 100 mEq of sodium is usually needed in a 24 hour period. In some of these patients, the urine potassium may drop to below 10 mEq per liter. Acidosis is known to disturb intracellular composition, as described many years ago by Van Slyke. These patients may have been losing potassium for a long time before they come for treatment; thus potassium replacement would seem essential.

The attempt to regulate the electrolytes in renal failure by raising the electrolyte level suddenly may be hazardous. I think it best to proceed slowly in trying to correct deficiencies. It is important to maintain a good calcium intake. This consists of removal of phosphate via the gastrointestinal tract and also prevents the danger of tetany, which may occur as the bicarbonate level is increased. It is advisable to raise bicarbonate to normal but not above.

V. THERAPY OF THE NEPHROTIC SYNDROME

A. Complications of Therapy

CHAIRMAN METCOFF: We had planned to talk about some of the complications of the nephrotic syndrome with and without steroid therapy, since we have not done this for a good many years. I would like to present briefly some data derived from nephrotic patients who have received steroid therapy since 1950, and from non-treated nephrotic patients observed since 1946. The data represent a ten year experience in our clinic at the Children's Hospital in Boston. During that interval Dr. C. A. Janeway and I have provided continuity in management. A very cooperative and hard-working house staff has, throughout these years, been alert and sensitive to the problems of nephrotic children. They have carefully recorded most of the observations which make this assessment possible The punchcards started by Dr. John James and I were brought up to date by Drs. John Knapp and Robert Schwartz during the past few months. They were good enough to send them out to me. I have made a preliminary sorting of the cards in order to present some of the more obvious complications. During the period January 1950 to July 1956, 153 children were treated with complete 10-21 day courses of either ACTH or cortisone, one or more times. The incidence of serious infections occurring during the course of therapy or within 24 hours after its cessation is indicated in Table 15. Complications such as convulsions were, to my surprise, much more frequent than were infections in the course of therapy. My clinical bias led me to think that serious infections were a frequent complication of steroid therapy. That does not seem to be the case. However, the series is heavily weighted because we were looking for infections in the patients, and either did not treat them with steroids or stopped such therapy should one occur. Moreover, all patients were on antibiotic therapy during hospitalization and/or therapy.

Hematuria occurred in 11 patients where it had not been present as a complication prior to therapy. Hypertension (blood pressure higher than 140/100) was among the most common of the complications attending steroid treatment, occurring in 34 children or 22 per cent of the patients under treatment. Hypertension was considered a contraindication to steroid therapy. Therefore, it was not present prior to therapy. It usually disappeared once the therapy was stopped.

DR. COOKE: Can you correct convulsions for hypertension? How many of that group of 16 did not have hypertension?

CHAIRMAN METCOFF: An appreciable number did not have hypertension. Some of them did.

TABLE 15

Complications during Steroid Therapy of Nephrotic Syndrome in 153 Patients (1950-56)

Infections	Number of patients	Per cent
Peritonitis	2	1.3
Cellulitis	3	2.0
Bacteremia	2	1.3
Genital Edema	8	5.2
CNS Complications		
Sensorium altered	4	2.6
Psychologic disturbances	5	3.3
Convulsions	16	10.4
Hematuria	11	7.2
Hypertension	34	22.2
Biochemical Complications		
Acidosis	15	9.8
Alkalosis	17	11.1
Hyponatremia	33	21.5
Hypokaliemia	40	26.1
Hypocalcemia	8	5.2
Tetany	6	3.9
Azotemia	47	30.7

DR. LANGE: Could we approximately know the dosage that was given? I know that has varied in cases.

CHAIRMAN METCOFF: The usual dose that we have used for ACTH is 150 to 200 units per square meter per 24 hours, and for cortisone it has been between 350 and 500 mg/M^2/day.

DR. RAPOPORT: May I ask a question about convulsions? We had a very baffling child who had cerebral symptoms, had a great deal of choking of her discs and somebody made a remark that he had seen some place that this was not uncommon in steroid therapy. It is the only one I have ever seen.

CHAIRMAN METCOFF: Choking of the disc?

DR. RAPOPORT: Somebody not concerned with renal disease, said "Gosh, you should have known this. This is common."

CHAIRMAN METCOFF: Only one of these children, as far as I know, had papilledema.

"Hematuria" requires definition. In most patients we do Addis counts. Hematuria refers to more than one million red cells per 12 hours. On casual, untimed specimens, more than 3 RBC/HPF in the centrifuged sediment was considered hematuria.

DR. BARNETT: These complications occur within 24 hours after stopping therapy?

CHAIRMAN METCOFF: During therapy or within 24 hours after therapy ceased. Usually if a complication arose therapy would be stopped.

DR. LANGE: You would stop therapy with any one of these complications?

CHAIRMAN METCOFF: Yes, if the more serious ones were noted.

DR. RAPOPORT: I would feel a little happier if you labeled these accompaniments rather than complications of steroid therapy.

CHAIRMAN METCOFF: Hyponatremia (serum sodium less than 130) occurred in 33 or approximately 22 per cent of patients under treatment. We use a "no-added salt" diet.

DR. COOKE: How often did water intoxication or symptomatic hyponatremia occur?

CHAIRMAN METCOFF: I think you refer to the relationship between hyponatremia and convulsions. A significant proportion of patients who had convulsions had hyponatremia, but I don't recall the number at the moment. In 40 patients or 25 per cent of the patients under therapy hypocalcemia (serum calcium less than 8 mg.%) was evident. Most children were receiving potassium chloride or acetate supplements P.O. Hypokaliemia (serum K less than 3.5 meq/L) occurred despite this. All of these children received the usual type of therapy and precautions that we have taken for many years. The events noted occurred in spite of these precautions. Tetany occurred in 6 patients with hypocalcemia. Azotemia (NPN over 50 mg.%) was the most common of all the complications that we observed. This occurred in 47 or 30 per cent of the patients under steroid treatment.

DR. HEYMANN: You did not discontinue the treatment?

CHAIRMAN METCOFF: Usually not.

DR. COOKE: Because of the fact that the NPN may rise significantly with steroid therapy, were there any changes in the creatinines?

CHAIRMAN METCOFF: Serum creatinine concentration or creatinine clearance?

DR. COOKE: Serum.

CHAIRMAN METCOFF: We have the measurements but I have not collated them.

DR. GOODMAN: We have done serum creatinines and BUN's on all our patients. In most cases the creatinines stay the same while the BUN's may often double.

DR. COOKE: Sure!

CHAIRMAN METCOFF: Table 16 shows the other side of the coin. It indicates the occurrence of these same manifestations in the same children when they were not receiving ACTH or cortisone therapy. The incidence of infection was much higher than during the periods when they were receiving steroids. This may be an artefact because they would not be included in the treated group unless they were without infection at the start of therapy. Moreover, no attempt has been made to adjust for the fact that the observations during steroid therapy encompassed a period of a few weeks, whereas those not during therapy represent several years of experience.

TABLE 16

Complications of the Nephrotic Syndrome NOT during Steroid Therapy in 153 Patients (1946-56)

Infections	Number of Patients	Per cent
Peritonitis	26	17.0
Cellulitis	40	26.1
Bacteremia	20	13.1
Genital Edema	32	20.9
CNS		
Sensorium	5	3.3
Psychologic	1	0.7
Convulsions	6	3.9
Hematuria	92	60.1
Hypertension	13	8.5
Biochemical		
Acid-Base	43	28.1
Hyponatremia	35	22.9
Hypokaliemia	18	11.8
Hypocalcemia	95	62.0
Tetany	1	0.7
Azotemia	77	50.3
Severe Diarrhea	33	21.5

There were fewer convulsions. Hematuria was commonly encountered. In support of Dr. Rapoport's comment, hypertension was less frequently encountered than during steroid therapy and acid-base difficulties of one sort or another were usually less frequently encountered than accompanying steroid therapy. Hyponatremia was commonly observed, even without steroid therapy. Hypokaliemia was less commonly encountered than during steroid therapy. Hypocalcemia and azotemia were more common during the periods when steroid therapy was not being given.

DR. McCRORY: Would you consider a serum calcium level of 8 mg.% abnormal if you only have three grams of protein?

CHAIRMAN METCOFF. I am very hesitant to answer that because I don't know the degree of calcium binding by proteins; on which fractions of the plasma proteins of nephrotics calcium is bound. The usual nomogram for ionized calcium may not be applicable in nephrotics. The only pertinent data on the subject, as far as I know is that of Hastings[1]. He showed that the ionized calcium in children with the nephrotic syndrome was decreased. To continue, severe diarrhea is a common event during the course of the nephrotic syndrome. I mention this only because it so often is a matter of chagrin to people treating the disease.

There were 18 deaths in this group of 153 patients during the course of steroid administration. These deaths were associated with complications indicated in Table 17. Some patients had several complications simultaneously. For example, a patient with peritonitis also had bacteremia. Azotemia was the most frequent accompaniment of the fatal episode. Hyponatremia occurred in two instances and was associated with convulsions in one.

TABLE 17

Complications Associated with a Fatal Episode During Steroid Therapy in 18 Patients

Infections	Number of Instances
Pneumonia	3
Peritonitis	2
Bacteremia	1
Electrolyte Disturbance	
Acid-base imbalance	1
Azotemia	11
Hyponatremia	2
Hypokaliemia	2
Cardiovascular	
Hypertension	4
Congestive failure	1
CNS	
Convulsions	1

DR. BARNETT: How many of those 11 patients with azotemia had reduced function before you started treatment?

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[1] McLean, F. C. and Hastings, A.B., The state of calcium in the fluids of the body. I. The conditions affecting the ionization of calcium. J. Biol. Chem. 108:285, 1935.

CHAIRMAN METCOFF: I could not answer that directly. Usually no patient with an NPN over 50 or with obvious evidence of renal insufficiency would have been subjected to a course of steroid therapy.

DR. COOKE: Did all 11 patients have their NPN's rise above 50 during the steroid therapy?

CHAIRMAN METCOFF: Yes, during the steroid therapy.

DR. LANGE: Would you still make the rule that you would not subject anybody to steroid therapy with an NPN over 50 or was this just an impression in the beginning?

CHAIRMAN METCOFF: It was our early impression, which we still maintain, that it is essential to get the patient in as good biochemical shape as possible before initiating therapy. Usually it was possible to reduce the NPN and correct other electrolyte disorders before we started therapy. Complications might arise during therapy but we almost never started in the face of almost certain complications. Four patients developed severe hypertension during the course of therapy and died during or immediately after the course of therapy. One patient developed congestive failure, and one patient had a convulsion. I believe the patient with the convulsion had both hyponatremia and hypertension.

DR. ZEIG: Have you broken down the causes of convulsion? You listed the total figure in the course of therapy.

CHAIRMAN METCOFF: I am sorry, I don't have the data available.

DR. BARNETT: Did you know the causes of any of the convulsions?

CHAIRMAN METCOFF: No. I think we could say quite clearly that we did not know the causes of the convulsions. We only knew events associated with the convulsions.

DR. COOKE: Did the convulsions cease with hypertonic electrolyte or with calcium, or lowering the blood pressure?

CHAIRMAN METCOFF: If tetany was present administration of calcium was usually helpful. If acute marked hyponatremia was present, we gave hypertonic saline. In general, we preferred not to use parenteral infusions to correct electrolyte dysequilibria in nephrotic children but rather attempted to correct the disorder with appropriate salts given by mouth when possible. When hyponatremia developed slowly, as it usually did, we rarely gave parenteral fluids. The convulsions usually were of very short duration. They would last for perhaps 8 to 10 minutes at the most, sometimes less than this, and we always attempted relief of any likely or obvious etiologic factor.

DR. HEYMANN: May I ask you one thing? I remember in our survey a few years ago we had 6 convulsion cases which were in part not explained. All 6 children died later on in renal failure. Do you have the same experience or not?

CHAIRMAN METCOFF: No. It is my impression that the convulsions in many of the instances where we have observed them were not associated with a bad prognosis. My feeling has been that convulsions might be more closely related to the type of therapy we were using in the management of the patient than to the renal lesion or prognosis. I would like to indicate the incidence of signs often thought to connote a poor prognosis in patients who are now in clinical remission. Clinical remission is defined as no edema and minimal proteinuria (i.e.: approximately 100 mgm. per 12 hours), a level of serum proteins within a normal range and serum cholesterols between 200 and 350 mg.%, all of these for at least six months to a year. By "Five year cure" is meant patients who have had no signs of the nephrotic syndrome either in blood, urine or symptomatically for 5 years, etc. The incidence of azotemia, hematuria, hypertension, acidosis and convulsions in these patients is given in Table 18. The patients are classified according to whether they ever received steroid or not. The number of patients who exhibited any of these signs is noted. Some patients showed more than one sign. There were 109 patients all told, 37 untreated with steroid and 72 who had been subjected to steroid treatment at some time during their course. At the present writing all appear to be well, have no evidence of renal inadequacy, are continuing to grow. Some of them have been well for 5 years or more.

TABLE 18

Incidence of Certain "Poor Prognostic Signs" in Patients with Clinical Remission or "Cure"

Sign	Occurrence of Signs		Total
	No Steroid Group	Steroid Therapy	
Azotemia	17	51	68
Acidosis	1	16	17
Hematuria	18	38	56
Hypertension	3	21	24
Convulsion	3	7	10
Total Number of Patients	37	72	109

DR. GOODMAN: We have done Addis counts on all our patients and have found at least a minimal hematuria, a few million red cells per 24 hours, in all patients before therapy. When Addis counts are not done, the number of cells per high dry field can change from time to time depending on how dilute the centrifuged urine sample may be, so I wonder how much --ress should be placed on the finding of hematuria unless Addis counts are done. I wonder how accurately one can assess renal function changes unless one rules out the BUN rise that comes from protein catabolism. I wonder if you could give us the totals of results as far as 5 year cures for the untreated versus steroid-treated patients.

DR. BARNETT: That BUN rise is associated with the reduction in clearance as measured by inulin, true endogenous creatinine clearance and everything else.

CHAIRMAN METCOFF. I don't understand what you mean, Dr. Goodman, when you say correct for the rise of BUN.

DR. GOODMAN: We have frequently seen patients who have quite a disparity between BUN and creatinine, and especially during steroid administration a further rise in BUN occurs without disturbance in serum creatinine. Obviously we would have to do inulin clearances to determine which is wrong. With relation to the normal creatinine and elevated BUN, we have assumed that an increase in protein catabolism occurred, producing a higher urea load. When getting steroid the patients eat more protein so that we have been more impressed with the unchanged serum creatinines and have minimized the significance of the urea elevation.

CHAIRMAN METCOFF: I don't think that it is fair to assume that there is increased protein catabolism throughout the period of steroid therapy. The effect of steroids on protein catabolism in the nephrotic patient is very short-lived and probably occurs during the first days of therapy as Barnett [2] suggested. I think our own data on nitrogen balance during ACTH therapy indicates that some children studied remain in positive nitrogen balance.

DR. GOODMAN: Aside from that point, since we don't limit the diet, the patients eat a lot more protein. That would increase the BUN by the protein load. There must be some component of the BUN rise which is due to these factors unless it is associated with a rise in serum creatinine.

CHAIRMAN METCOFF: A decrease in urea clearance may be the essential factor, if urea clearance parallels inulin, creatinine or thiosulfate clearances.

DR. GOODMAN: I suppose I should not say this. The people who did the clearances are not here. Jack Orloff and MacKenzie Walser, who did inulin clearances in these patients, did not find a reduction on steroid. In most cases the clearances rose.

DR. BARNETT: Adults or children?

DR. GOODMAN: Adults.

DR. BARNETT: If you look in our paper [3], a number of patients had inulin, PAH clearances done. There was almost consistently reduction of clearance during the time they were on the steroids, even though they started with a normal clearance.

CHAIRMAN METCOFF: I believe we also reported this at these meetings four or five years ago [4].

DR. GOODMAN: Maybe adults are different from children.

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[2] Barttler, F. C., Forbes, A. P., and Albright, F., A comparison of the effects of ACTH in panhypopituitarism, ovarian agenesis and acromegaly. Proc. of 1st Clin. ACTH Conf., J. R. Mote, Ed., Blakiston Co., Phila. 1950.

[3] Barnett, H. L., Forman, C. W., McNamara, H. and McCrory, W., the effect of adrenocorticotrophic hormone on children with the nephrotic syndrome. II. Physiologic observations on discrete kidney functions and plasma volume. J. Clin. Invest. 30:227, 1951.

[4] Fourth Annual Conference on The Nephrotic Syndrome, J. Metcoff, Ed., 1952.

CHAIRMAN METCOFF: Children have a striking reduction in clearance during the first three or four days of steroid therapy. Perhaps the results you describe, Dr. Goodman, refer to the usual post-therapeutic rise in clearance.

The data on status which you requested earlier, Dr. Goodman, are given in Table 19. Obviously insufficient time has elapsed to see many entries in the "cure" column of steroid-treated patients.

TABLE 19

Current Status (June, 1956) of Nephrotic Children (1946-1956) Relative to Steroid Therapy

Status of Patient	Number of Patients		Total
	No Steroid	Steroid	
5 year "cure"	9	1	10
Clinical remission	33	71	104
Dead	38	40	78
Lost to followup	16	14	30
Total	99	159	258

DR. COOKE: These events compared in Table 19 are not going on simultaneously. This is just comparing several years ago with more recent findings?

CHAIRMAN METCOFF. To some extent, yes, but actually there has been an accumulation of quite a few patients recently who have not been treated with steroids. There are many factors which dictate withholding of steroid treatment in particular patients. Any statistical classification is being weighted at both ends of the scale. Some patients are either too badly off to be subjected to steroid therapy or the physician feels that they have too minimal involvement to be subjected to steroid therapy. A significant number of patients in each group have died and the present status of about 12 per cent of the total group could not be ascertained.

DR. GRIBETZ: In the patients with the five year clinical cure, how many had proteinuria as an isolated finding? Do you sort of eliminate that as a cure if they still show it?

CHAIRMAN METCOFF: Yes, they would be considered clinical remission, not cure.

DR. GRIBETZ: Do you have an idea how many children still show an isolated finding?

CHAIRMAN METCOFF: Not at present.

DR. GRIBETZ. It is interesting that a majority of the patients that Dr. Jerome Kohn has followed, some for as long as 30 years, have proteinuria despite the fact that they are clinically well, active, and have normal blood chemistries and renal function studies.

DR. KOHN: Seventy-five per cent of them, in fact.

CHAIRMAN METCOFF: The ones we call five year cures do not have proteinuria on any specimen that we have examined. Otherwise they would not be so classified. In most instances Dr. Kohn's patients would have had qualitative tests for proteinuria?

DR. GRIBETZ: That is right.

DR. BARNETT: Out of 65 patients followed by Dr. Van Slyke from 5 to 25 years there were 23 who had no proteinuria and no other sign. He even eliminated some from this "cured" group who had no protein but who continued to have supernormal clearances.

B. Survival Rates and Steroid Therapy

CHAIRMAN METCOFF: I should like to summarize the adjusted mortality rates by yearly intervals observed in our group at Children's Hospital in Boston. All patients who received steroid therapy of any sort or at any time during their course are included in the "steroid-treated" group. All others are included in the "no steroid" group. A life table calculation, similar to that employed by Dr. Riley, was used.

There were several difficulties encountered in analysis of the data at this time. Outstanding among these was the necessary withdrawal of patients from analysis because of the arbitrary cutoff point in October 1956 (Table 20). This particularly penalized the steroid treated group, since the short followup period after onset and treatment strikingly reduced the sample size. The future course of the withdrawn, but living, patients cannot be assessed. Withdrawals from the untreated group were considerably fewer and decreased with increasing time of followup, largely because many of these patients were seen in the period prior to 1950, and by 1956 their course was determined. Most of the reduction in sample size in this group resulted from deaths.

An adjusted mortality table was constructed embracing a five year experience. Although the experience was continuous in each group, it was not generally simultaneous. For example, the majority of the "no steroid" patients entered the group before 1950; the majority of the steroid-treated group entered after that date. Since the data were handled on the basis of a 12 month interval, the adjusted death rates (qx) were calculated as observed deaths per interval (dx) divided by the sum of living individuals at the beginning of the interval (lx), and those entering during the interval (nx), minus those withdrawn during the interval (wx).

: $qx = \frac{dx}{lx + nx - wx}$. The results are shown in Table 21. The mortality in the no steroid group was significantly greater during the first two years following onset ($p = < 0.05$ and < 0.02). The number of withdrawals in the steroid treated group was too great to evaluate the data after the first three years of observation. Evaluation of this same experience on the basis of a six monthly interval where

$qx = \frac{dx}{lx + 1/2nx - 1/2\,wx}$, yielded results shown in Table 22.

TABLE 20

Withdrawals* from Analysis as Per Cent of Initial Yearly Sample

Year	R_x Group	No R_x Group
1	18.8	9.9
2	16.0	10.4
3	18.6	10.9
4	19.5	6.7
5	23.1	5.1

*Total numbers lost from followup: R_x = 14, No. R_x = 16. Most withdrawals occasioned by limit of observation period from onset to October 1956.

TABLE 21

Annual Adjusted Death Rates Nephrotic Children

Years Observed	Per Cent Mortality	
	Steroid R_x	No Steroid R_x
0-1	5.0	13.4
1-2	7.6	21.7
2-3	12.7	12.2
3-4	10.3	7.1
4-5	7.5	2.7

TABLE 22

Adjusted Mortality Nephrotic Children by Six Monthly Intervals

Interval	Adjusted Group Size		Death Rate Per Cent	
	Steroid	No Steroid	Steroid	No Steroid
0-6	60	37	5.0	19.2
7-12	118	68	2.6	5.9
13-18	113	69	3.5	11.7
19-24	104	61	3.9	11.6
25-30	93	53	5.4	3.8
31-36	79	47	6.4	8.5

Obviously it is necessary to follow the steroid-treated children for another 2-3 years before firm conclusions can be reached. Perhaps Dr. Janeway and Dr. Robert Schwartz will be able to do so. It would seem, however, that courses of steroid therapy

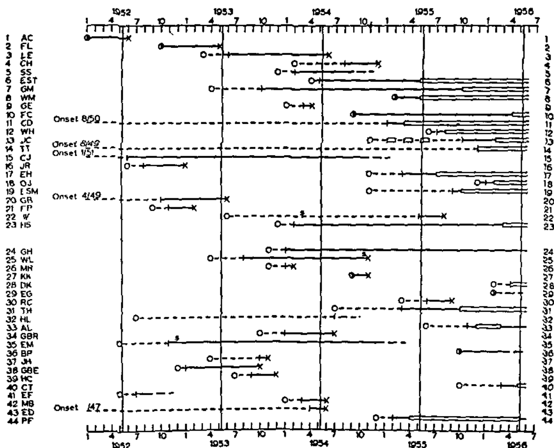


Fig. 37. Outcome and treatment in 44 unselected cases of the nephrotic syndrome (see text)

o - onset. x - death. s - surgical intervention.

----- period of illness not under observation.

entry into series and period of observation with conservative treatment.

observation with cortisone + oral penicillin.

(Reproduced from Squire, et al., *Brit. Med. Bull.*, in press.)

even without maintenance therapy may favorably influence the mortality rate in the nephrotic syndrome. Our experience, therefore, appears to differ from the conclusions reached by Drs. Riley and Fertig, previously reported to this group[6].

If we may go on, it might be of some interest to those of you who don't know about the types of therapy which are used in the British Isles to hear from both Dr. Blainey and Dr. Arneil regarding their management of the nephrotic syndrome. Dr. Blainey!

C. Steroid Therapy of the Nephrotic Syndrome in Britain

DR. BLAINEY: Figure 37 shows the data on 44 cases we have studied in Birmingham[7]. We have treated our cases with low dosage of cortisone for long periods continuously (75-150 mgm. per day for the average patient). We have used ACTH once only as the patient showed a considerable rise of blood urea nitrogen which was shown to be due to a great increase in urea production and excretion. The resulting negative nitrogen balance took about 6 weeks to be replaced and this seemed highly undesirable in an already protein depleted patient.

The first 23 cases in the table are those with primary renal disease as a cause of their nephrotic syndrome: the remainder have associated conditions such as primary amyloidosis, myelomatosis, lupus nephritis, renal vein thrombosis, etc. The response to therapy has been judged by the daily loss of protein in the urine and more recently, by following the level of serum complement[8]. While this table is a very nonstatistical method of analysis of the data, it does, I think, show that the early results of cortisone in the adults are encouraging, especially in primary renal disease. The mortality does seem to have decreased during the past year since we have used cortisone and there is no doubt whatever of the reduction in the proteinuria and rise of serum albumin with diuresis in many cases. Some patients still have proteinuria after long periods on steroids, and we have continued to treat these without ill effects.

The patients in the second group have shown a less satisfactory response to cortisone, and in several cases of myelomatosis and lupus nephritis, there does not seem to have been very much effect, at any rate in the dosage that we have used.

It is obviously too early to assess the longer term effects of this treatment and what I have been saying must be rather in the nature of a preliminary communication. We are certainly going to follow all these cases for a long time to see what does happen.

DR. HEYMANN: Your patients are chiefly adults?

DR. BLAINEY: Almost entirely adults.

DR. LANGE: Were there cases with what we call Kimmelstiehl-Wilson disease treated with cortisone?

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[6] Riley, C. M., Davis, R. A., Fertig, J. W. and Berger, A. P., Nephrosis of Childhood: Statistical Evaluation of Adrenocortical-Active Therapy, J. Chronic Dis. 3:640, 1956.

[7] Squire, J. R., Blainey, J. D. and Hardwicke, J. Brit. Med. Bull. (in press).

[8] Lange, K., Slobody, L., and Strang, R., Pediatrics, 15:156, 1955.

DR. BLAINEY: We did not have any diabetic patients in this series. Those with primary amyloidosis and myelomatosis did not apparently show any response to steroids in the doses that we were giving them.

CHAIRMAN METCOFF: Thank you. Dr. Arneil, would you care to report on a Scotch experience?

D. Therapy in Scotland

DR. ARNEIL: I must apologize if my style seems telegraphic. The first data I would like to show is the interpretation of the effect of the advent of hormone therapy on the prognosis of cases of nephrosis in our area[9, 10]. It is very difficult to make a valid assessment; we are unfortunate in that the number of cases is small but fortunate in the fact that in a comparatively tight little island a more complete followup is practicable. Table 23 represents 56 consecutive cases of nephrosis.

TABLE 23

Current State of 56 Cases of Nephrosis Three Years After Treatment

	Hormone Group	Control Group
Asymptomatic	14	11
Continuing	6	4
Dead	8	13
Total	28	28

The groups are comparable in that the age distribution is similar; they were all treated in hospital by the same physicians, and the sulfonamides and penicillin were available for the treatment of infection when it arose. In one group, however, steroid therapy was available. The results at the point three years after treatment (which virtually coincides with the onset of edema), are laid out. These have been divided into three groups, the first consisting of patients who are free of signs or symptoms of the disease (asymptomatic), the second in whom at least one symptom or sign persists (this is generally minimal albuminuria), and the third group, of those who had died. You see that no spectacular improvement has resulted from this short term treatment with ACTH and cortisone. Nevertheless, the number dead is less in the hormone group, and more than this 50 per cent of these children are asymptomatic. The interpretation of these results seems to be that we have prolonged the life and accelerated the tendency to remission in these cases. All these cases were what might be termed "pure" nephrosis in that the etiology was unknown and no specific illness or injury preceded the attack. I thought it might be of interest to you to compare these figures with those of your own series. The form of treatment which we now employ is as follows, and differs materially in several respects from the methods generally in use over here.

[9] Arneil, G. C., Hormone Studies in the Treatment and Investigation of Nephrosis, Scot. Med. J., 1:275, 1956.

[10] Arneil, G. C., Prednisolone Treatment of Nephrosis, 1:409, 510, 1956.

As soon as diagnosis is established we begin treatment with prednisolone, or delta-cortril, as it is called here. This is very similar to prednisone, with which it may well be confused, but it is far from proven that the action of the two is identical, particularly in relation to the production of hypertension and peptic ulceration. The dosage is started at 60 mg. daily and scaled down over a period of 40 days. No added potassium is given during this period and the diet contains less than two grams of sodium daily. The consistency of results which have been obtained has been such as to encourage us greatly, with many reservations that luck has played a considerable part. Not only has edema been removed but the albuminuria has been decreased or abolished and the serum biochemistry returned to normal with rapidity and regularity which are materially better than the results obtained with cortisone or ACTH in this center.

We do not give antibiotic cover either during prolonged cortisone maintenance or active treatment. This is because no significant increase in the number or severity of intercurrent infections has been noted by myself or Professor Graham. In any case, when the gamma globulin level is restored to normal and the period of anasarca so brief the need for such cover seems very dubious. Any infection which does arise is at once treated with antibiotics. It seems to me that the promiscuous prescription of antibiotics has vitiated the good results claimed for long term continuous or intermittent hormone treatment since no control group receiving antibiotic treatment is usually arranged.

DR. McCRORY. Are your patients isolated in a hospital setting during the whole course of therapy?

DR. ARNEIL. They are kept in hospital for a much longer period in Great Britain because no economic stress is placed on the parents by this procedure. The average stay of our cases is about three months; most of this time will be spent in a ward with perhaps twelve other patients and adequate teaching facilities are of course provided. They are not strictly isolated but removed from obvious sources of infection and any illness occurring is promptly treated.

DR. RAPOPORT: You have treated them for 40 days consecutively. Have you rendered every one free of protein in his urine?

DR. ARNEIL. No. Not every case. First of all one boy who had nephrosis for five years was treated with cortisone and ACTH. He was still edematous with gross albuminuria and thus was so treated. The albuminuria at once fell below 250 mg. daily, and all other symptoms and signs disappeared. Thereafter we left well enough alone. In two cases albuminuria relapsed shortly after treatment was omitted and required to be retreated with a 40 day course. In both instances this was apparently completely successful. Out of 12 cases treated 4 have slight degrees of albuminuria persisting on a very short term followup.

DR. RAPOPORT In your patients the majority cleared their albuminuria. What is the longest time it took you in this 40 day period to clear them? I have a reason for asking that. The longest one we have in our series is 18 days. We did not carry treatment beyond 28 days.

DR. ARNEIL: In the neighborhood of three weeks. I really can't give an accurate number of days. I should think three weeks.

DR. COOKE: Your statement about that I think is somewhere and did not agree with our experience. We had one case that had albuminuria persist for 48 days on therapy and then the albuminuria disappeared.

DR. KRETCHMER: Does the cholesterol diminish at the same time as the albumin?

DR. RAPOPORT: It comes down.

DR. COOKE: I think Dr. Guild has a case that beats mine.

E. Other Experiences with Therapy

(1) Dr. Guild

DR. GUILD: I have one in whom it took as long as 2 months, 63 days to be exact, for the albuminuria to disappear.

DR. GOODMAN: The same dose of steroid?

DR. GUILD: The child received 200 mg. of cortisone daily for 2 months before the urine became clear.

DR. McCRORY: How much steroid did you have your patient on, Dr. Cooke?

DR. COOKE: 200 mgm. of cortisone in a child who was a year and a few months of age. Your case is the longest I ever heard of, Dr. Guild. How old and how large a dose? (Ed. But cf. report of Dr. Arthur Merrill, VI. Ann. Conf. Neph. synd., 1954)

DR. GUILD: This boy was 6 years of age and had had nephrosis for 3 1/2 months when he came to us. He had been given cortisone sporadically during the preceding 2 months: 150 mg. daily for 12 days while in a local hospital and then 200 mg. daily for 2 days out of every week at home, with only slight response. During the week prior to admission to Harriet Lane his dose had been increased again to 150 mg. daily without improvement. After a 10-day period of observation in Harriet Lane without steroid, the boy was placed on cortisone in a dosage of 200 mg. daily, which was continued without interruption for 2 1/2 months. At the end of exactly 2 months the albuminuria disappeared; after 2 more weeks, during which the urine remained clear, the cortisone was gradually decreased with resultant reappearance of some albumin in the urine but without recurrence of signs or symptoms of the disease. Now, 2 years later, on 40 mg. of cortisone daily, the boy is still in remission with only an occasional trace of albumin in the urine, normal blood pressure and completely normal blood chemistries and kidney function tests. Further gradual withdrawal will continue and it is expected that the boy will be off cortisone within the next few months, for the first time since the 200 mg. course was started.

Several other patients have received as much as 200 mg. of cortisone daily for much longer periods than has this boy, but without complete disappearance of albumin at this dosage level, even though otherwise in remission. They have been maintained for many months at this level because, on every attempt at lowering the dose, symptoms have recurred, and it has seemed important to keep the disease under control, at least to the extent of suppressing the clinical manifestations.

CHAIRMAN METCOFF: Are there any other particular comments in relation to therapy?

DR. BARNETT: I would be interested in hearing any experience with very much larger doses of hormone that I understand are being used in a few places. I wonder if anyone here knows any more than the secondhand reports I have had.

DR. GOODMAN: I think Jim Baxter can tell about our experience.

(2) Dr. Baxter

DR. BAXTER: I think we have ordinarily used doses in the same range as that used by most others here. We routinely start patients now, whether they are adults or children, on 40 mg. of prednisone (or an equivalent dose of hydrocortisone) daily, and continue this dosage for something like a month. This dosage was chosen because of our observation that smaller doses were ineffective in some cases in which remissions were subsequently obtained with 40 mg./day. When a good response is obtained, the dosage is tapered off after maximum improvement has occurred (usually after about a month) and stopped. In cases where improvement has not occurred within about 3 weeks, we have often then increased the dosage. We have given a number of patients 80 mg./day for 2 to 4 weeks, and one adult received 160 mg./day for 2 weeks, following several ineffective courses with lower dosage levels. We have no clear-cut examples of patients having good responses at higher dosage levels where 40 mg./day was ineffective. There have been a few examples, particularly in adults, of gradual improvement over a period of months not definitely related to the courses of steroid therapy, in which the higher dosages may have played some part. On the whole our experience with doses above 40 to 60 mg. per day has been disappointing. It probably is true that higher dosages are more likely than lower dosages to cause "symptomatic" diureses (unaccompanied by any fundamental change in the disease) on withdrawal of the steroid.

DR. GOODMAN: We have been impressed by the results in lupus erythematosus. In Los Angeles, Dr. Dubois has administered grams of cortisone daily to produce remissions in some cases. He is dealing with patients in whom production of remission is a life-saving procedure. Our problem has been to arrive at a compromise between the possible danger to the patient from prolonged high dosage of steroid and the possible benefit which we might hope to see. We have not yet seen a clear-cut example of response to a higher dose in a patient who did not respond to 40 mg. of prednisone.

CHAIRMAN METCOFF: You may have noted that there is no particular discussion this year of the combined accumulated life table statistic because Dr. Riley has recently published the material available to him. Nothing further would be gained by presenting it again at this time, he felt. We hope that we may have further information on the combined study -- perhaps at next year's session.

DR. ARNEIL: In the neighborhood of three weeks. I really can't give an accurate number of days. I should think three weeks.

DR. COOKE: Your statement about that I think is somewhere and did not agree with our experience. We had one case that had albuminuria persist for 48 days on therapy and then the albuminuria disappeared.

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in plasma and we sought to find as delicate a method of estimation as possible. The method employed used a young 220 gram rat, under anesthesia with tracheotomy performed and then an arterial manometer inserted and an intravenous drip started whereupon our biological solutions were tested against standards such as vasopressin and later on angiotonin. At this stage we took plasma from the patients who had shown the greatest amount of peptiduria, (that is in acute glomerular nephritis), separated it with heparin immediately, chilled it and tested this plasma and normal controls to see if any difference could be found. Initially none was found, but fortuitously this plasma was exposed at room temperature for 12 hours and a perceptible difference noted in the pressor activity of the two types of plasma, that from the normal and that from the hypertensive patients. This property seemed to diminish as the patient's illness regressed. This property was subjected to investigation and we went on to what amounted to a physiological study in an endeavor to define this pressor factor. It is inducible in normal plasma much more readily at 37°. Plasma retained at that temperature will acquire marked vasopressor activity. Activity comparable to 200 cat units of angiotonin (Eli Lilly unit) per 100 millilitres is acquired in about 12 hours and one can draw a very nice curve showing the acquisition of this property. An attempt was made to define the substance. The method of testing obviated a number of substances; for instance, the adrenalines were ruled out, most organic amines, and the vasotonins of which serotonin is the best known. Vasopressin was ruled out by specific chemical test with sodium thioglycolate and the activity was then compared to angiotonin (as you call it), the product of hypertensinogen and renin. It seemed to us if this substance developed in plasma at body temperature, it might be some enzyme system which produced it. At first we could find no difference between the substance and angiotonin but later on as our tests became more accurate we were able to note several points. There were minor differences in the duration and pattern of the curve produced; on occasions we could eliminate the reaction to the plasma factor but not to the angiotonin. This was done fortuitously at first by air embolism. We then tested the substances in a different preparation, in a preparation of isolated guinea pig ileum, and a very marked difference in the pattern produced by the two was noted. This is the method suggested by Gaddum for differentiating two pressor substances. They should produce similar patterns in different preparations. It is interesting to note that this qualitative test which we used was proved afterwards to be more delicate than any other test which had been used for angiotonin and is now, I believe, becoming a standard method of testing for it. We failed to appreciate this. The possibility that stored plasma in the blood bank might have developed this pressor activity attracted attention and after having obtained specimens such activity was verified[12]. In fact, without any further incubation, blood bank plasma has marked activity as compared to normal plasma. The actual isolation of this substance has not been achieved. I don't know what it is. I have not yet met anyone else who does, but that is the stage that we have advanced to so far.

CHAIRMAN METCOFF. This pressor substance is in plasma, not whole blood, or is it present in both?

DR. ARNEIL: It is present in the plasma of the whole blood or plasma.

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[12] Arneil, G. C., Pressor Activity in Stored Plasma, Arch. Int. Pharmacodyn. Ther., 1956.

Now we have a few remaining minutes and Dr. Arneil has done some very interesting work in relation to pressor factors, isolation of pressor factors from plasma in patients with nephritis, I believe. Is this correct? If Dr. Arneil perhaps would summarize his work for us we would appreciate it a great deal. Before he does, however, I would like to comment in closing this session that we are particularly indebted to Drs. Blainey, Arneil, Brun, Josephson and others for the privilege of having you attend as representatives from other parts of the world in order that we may profit by your experience and by a different point of view. I want to thank all of you for your participation and I want to thank our host, Dr. Barnett, for setting up the conference so beautifully for us.

VI. Summary of Work with Pressor Substances

DR. ARNEIL: Perhaps I may be permitted to say a few words of thanks on behalf of the guests. The benefits which have accrued to us far exceed our contributions and we would like to thank you all.

In telescoping this topic, the result of five years' work into five minutes, I hope the remarks will not be too telegraphic for ready understanding. This project originated from our endeavor to shed some light on the mechanism of diuresis provoked by cortisone and ACTH in nephrosis. Some years ago, Barlow reported finding an antidiuretic activity in the plasma of nephrotic patients and this stimulated us to look at the problem from this angle. The tack on which our initial course was laid derived from the investigation of a peptide which Dent had described as being present in the urine of nephrotic patients. This was found to be constantly present in variable amount and we initially investigated other conditions characterized by edema and oliguria, namely, pre-eclamptic toxemia and the acute stage of acute glomerular nephritis[11]. The first thing observed was that, in fact, similar peptide was present in the urine of these patients. An attempt was made to find some explanation for this and to tie up a pituitary dysfunction. Incidentally, this is old history, and we are not holding this theory still. This peptide was analyzed as far as we could, and contained 12 amino acids. We mapped out its chromatographic behavior and its electrophoretic behavior. It was then sought in a number of biological fluids, amongst which were commercial posterior pituitary extracts. In all of these a similar peptide was noted. When I say "similar" it behaved in an identical fashion chromatographically and electrophoretically and contained the same 12 amino acids. This excited us greatly and we got hold of some volunteers, loaded them with water, initiated diuresis and then nicotine antidiuresis and examined their urine before and after antidiuresis and found peptide had appeared in their urine. These peptides were then subjected to biological investigations as antidiuretic substances and as a vasopressic agent, and right away, differences were apparent. The pituitary peptide, if I can call it such, was the only part of the pituitary extract which was active. It was highly active both as a vasopressor substance and as an antidiuretic. Tested in rats the urinary peptide was not active as a vasopressor or as an antidiuretic substance and appeared to produce toxic symptoms in rats. From this, arose a theory which led us to something much more interesting. It was thought that there might be some degradation before excretion. Vasopressor substances were therefore sought

[11] Arneil, G. C. and Wilson, H. E. C., Isolation of pituitary Antiduretic Peptide and similar Urinary Peptide. Lancet, 1:568, 1953.

projector. Some have sat and talked with him during the two days of the meeting. It is the hope of the Foundation that with their growth the support of this conference will also increase.

With these words, I shall say goodbye, and thank you again for coming.

DR. BARNETT: Is it active in man?

DR. ARNEIL: I do not know. In order to test, this should require a delicate continuous method of recording blood pressure and none is yet sufficiently delicate to do that. Is there any such method available in this country?

DR. BARNETT: There are a number of them.

CHAIRMAN METCOFF: The Hamilton recording manometer will do it.

DR. HEYMANN: Do you know of any relationship between your work and the work of Zipf, a German pharmacologist, who in 1931-32 worked on the pressor substances observed in rabbit serum after the blood had coagulated. I remember that he believed he had isolated or identified chemically the substance responsible for the activity.

DR. ARNEIL: The Australians have described a factor in human blood which develops when blood is allowed to stand and clot. Reid and Bick in Australia described this. It is not the same substance. It is related to breakup of platelets. If you remove the platelets beforehand, you eliminate the factor. It does not apply to these substances.

DR. LANGE: Lack of a depressor substance or deterioration of pressor substance?

DR. ARNEIL: I have tried to telescope things too much. It could be a lack of depressor. We can only call it a vasopressor factor at the moment. That is what we have called it, not a substance.

DR. COOKE: All bank bloods that you have tested have this?

DR. ARNEIL: So far, yes, about a dozen, all prepared by the same method.

DR. KRETCHMER: Is there a possibility that amines are causing the vasopressor effect?

DR. ARNEIL: I think so. Amines have been tested in this rat preparation treated with dibenamine, (which is ganglion blocking for both adrenergic and cholinergic substances) and none of those that we have tested have produced pressor activity. In fact they tend to be vasodepressor, particularly serotonin in this preparation. I don't know what it means. It is an interesting observation and that is all I can say of it so far.

CHAIRMAN METCOFF: Thank you very much, Dr. Arneil.

Dr. Barnett, would you like to close?

DR. BARNETT: I would like to say again that it has been a great pleasure for us to have you here and I welcomed the opportunity for those of you out of town to see our new place for the first time. I would like to mention that for those of you who are not aware of it that the publication of these transcripts of the meetings is supported by the National Nephrosis Foundation. Dr. Henry Kaessler, the President of this Foundation, whom we have had the pleasure of having with us, has been sitting over next to the

projector. Some have sat and talked with him during the two days of the meeting. It is the hope of the Foundation that with their growth the support of this conference will also increase.

With these words, I shall say goodbye, and thank you again for coming.